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FLOW INJECTION ANALYSIS
A PRACTICAL GUIDE

Bo Karlberg and Gil E. Pacey

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TECHNIQUES AND INSTRUMENTATION IN ANALYTICAL CHEMISTRY

FLOW INJECTION ANALYSIS

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PREFACE

"Flow Injection Analysis should not be explained. It ought to be demonstrated".

"Flow Injection Analysis is a tool to perform chemistry".

These are the two guiding principles of our Flow Injection Analysis (FIA) experience. During all the years of demonstrating, lecturing and using FIA it became clear to us that a practical guide to FIA was needed. It is not really important to the practicing FIA analyst whether tanks in series or random walk is the correct theoretical model for FIA. What is important is how flow rates affect sample dispersion and how to improve selectivity of a method.

At first glance at a commercial FIA system or first reading of most FIA literature, it would appear that designing of an FIA system is trivial. However, the choice of components and material and the overall geometry of the system is critical to achieve the advertised advantages of FIA.

The time scale of FIA has reintroduced the necessity for the knowledge of chemistry in the method development process. In fact, new methods which are "impossible" in batch mode or in air segmented flow systems can be developed using the FIA principle.

The basic outline of this book closely follows the schedule of the FIA workshops which we have tought. Many of the workshop students have become the most active developers of clever FIA methods. This ability is clearly related to a "nuts and bolts" understanding of FIA.

The book has two sections, FIA principles and FIA applications. The application chapters show examples of complete methods which demonstrate the versatility of the FIA technique. A bibliography is included. This is a selected bibliography where Ph.D. theses, uncommon and less accessible journals, reports and non-English language contributions have been excluded. The bibliography is completely indexed by analyte, area of application and detection technique.

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CHAPTER 1

INTRODUCTION

1.1 MODERNIZATION OF SAMPLE/SOLUTION HANDLING TECHNIQUES

Until the early parts of this century research scientists involved with making analytical measurements had very limited and primitive equipment at their disposal. Light sources were flames and the sun. Most wavelength dispersion/discrimination was performed using filters or prisms. The detectors, in many cases, were the eyes or eventually photographic films. The readout device was a person capable of evaluating the observed signals. Data evaluation was again a person. The sample was collected by a person and filtered, diluted, and in general handled by a person using volumetric glassware, like pipets and volumetric flasks. All measurements were slow, tedious, and required a great deal of skill on the part of the analyst in order to ensure precise and accurate results.

Fortunately for us, the scientific and engineering communities have developed considerably improved ways to carry out the aforementioned measurement steps. Light sources now include lasers capable of extremely well controlled power, spectral wavelength, coherence, and reproducibility. Wavelength dispersion is carried out using laser blazed gratings and holographic gratings. Detectors range from single photodiodes to diode arrays capable of collecting the entire spectrum that is desired. The readout devices go from simple chart recorders to computers. The collection, evaluation, and the report itself is handled by the computer, hopefully with our assistance.

But strangely enough the sample handling step of analysis is, in many laboratories, still at the flask and pipet stage. Modernization of sample handling has proceeded remarkably slow. Skeggs realized the need to improve the efficiency of chemical analysis (1). He noted by the 1950's that at the present rate of development, fields such as clinical analysis would eventually demand such high volumes of analytical determinations that the laboratories would not have sufficient personnel to make those determinations. The segmented continuous flow analyzer was developed to overcome this sample throughput problem. This type of analyzer has been commercially refined by Technicon and is marketed under the tradename Autoanalyzer. Several thousands of these units have been sold.

However, total modernization of the sample handling step of analysis requires a reduction in time and improvements in the types of sample handling steps that can be automated such as matrix modification and dilution. The Autoanalyzer, in most situations, must allow the chemistry to proceed to steady state and has limited flexibility, especially in terms of timing, thereby making kinetic based measurement impractical. A new technique for automated sample handling was needed. Flow Injection Analysis (FIA) is such a technique. In FIA the sample is very reproducibly controlled in the flowing stream. Steady state measurements are no longer necessary because of this high reproducibility based on the basic FIA properties of reproducible timing, sample injection and controllable sample dispersion. This allows for significantly reduced analysis time. With FIA, matrix modification and kinetic based measurements become practical.

The sample handling step of chemical analysis is finally modernized. A sample under FIA conditions can be manipulated in every way imaginable. This flexibility gives the analyst a wide range of conditions to carry out the desired chemistry whether in the laboratory or the process control stream.

1.2   AUTOMATED ANALYSIS IN GENERAL (WHERE, WHEN, AND WHY)

The arguments for automation are quite varied. Automation is commonly employed to reduce the manpower cost of a laboratory operation. The need for high sample throughput is another major reason for automation. Additionally, automation equipment is used in situations which are too hazardous for human exposure. For example, the measurement of analytes in radioactive samples is more easily carried out under automated conditions, thereby eliminating a large amount of the concern over occupational safety. A fourth example of automation is in the process stream where the environment of the measurement is poor but the need for accurate control essential.

Until the advent of FIA the automated system was seldom used to improve the chemical method being utilized. Automation was simply a cost effective and safe way to carry out the necessary determinations. Under FIA conditions the biggest single problem that faces an analytical chemist can be addressed; the sample matrix. In the real world the samples that are received seldom are in a clean, easy to handle matrix. Usually the sample is in an incredibly difficult matrix that not only may contain potential chemical interferents, but also may exhibit properties such as turbidity, viscosity, or salt content that simply make the usual standard method of determination essentially useless.

By handling the sample matrix in a reproducible manner, the use of solution based chemistries in the laboratory or process streams is a reality. For example, the matrix can be handled by a selectivity enhancement technique such as gas diffusion or a simple kinetic procedure allowing direct measurement of the analyte of interest.

Flow Injection Analysis is a tool for the analyst to use. We must remember, however, that a complete and accurate understanding of the chemistry is always essential to the successful application of any automated method. FIA can be used to control and/or modify the known chemistry in order to increase selectivity, sensitivity, and improve precision and accuracy. The application section of this book will give several examples of the way in which FIA has been used to improve the chemistry and the subsequent analytical measurement.

1.3 COMPARISON OF FIA VERSUS HPLC, SEGMENTED CONTINUOUS FLOW ANALYZERS AND ROBOTICS

In the early days of FIA most people tried to compare FIA to high performance liquid chromatography (HPLC) and to the segmented continuous flow analyzers. The comparison to HPLC was unfortunate since many people began to believe that there were many similarities, when in fact the similarities are few. To start with, the objectives of FIA and HPLC are very different. The objective of chromatography is to separate and detect several constituents of a sample, while FIA is usually used to create a detectable signal on only one species. HPLC needs high pressure, about 1000 psi, to push the sample through the column. FIA seldom has pressures above 7 psi. HPLC does not have the inherent reproducibility or controllable sample dispersion that FIA exhibits. While both techniques utilize sample injection and FIA will sometimes have small columns, it must be stressed that HPLC is a separation technique; at this time FIA is incapable of carrying out high resolution separations. Thus, they are two distinctively different techniques.

The comparison to segmented continuous flow analyzers is a fair one. The fundamental operational difference between the two types of systems is the segmentation. For some time it was assumed that air-segmentation was an absolute necessity. Segmenting allowed the identity of each individual sample to be preserved. However, injection of the sample accomplishes the same objective making segmentation unnecessary. Additionally, the segmented continuous flow analyzer is normally operated under steady state chemical conditions. Set-up of the system requires a great deal of time and effort. In FIA, steady state conditions are unnecessary because of the reproducible timing, and the controllable sample dispersion.

The primary drawback to the air-segmented systems is the bubble. The air bubble is compressible, thereby creating pulsations in the flowing stream. For most detectors the air bubble must be removed before the sample passes through. In addition, precision of bubble size has been difficult to control. This variation in bubble size adds to the irreproducibility of the system. If electrochemical or other static sensitive detectors are

used, the segmented system, which builds up static charge, will present severe problems in terms of baseline stability. Other concerns include the variation in pressure and flow velocities and the fact that conversion or selectivity enhancement techniques are not easily incorporated into these systems.

By eliminating the air bubble many of these concerns are removed. The most important advantages gained by elimination of the air bubble are:

1. Introduction of a reproducible sample volume into the system by injection.

2. Reproducible handling in the system with reduced pulsation.

3. No predetector bubble separation is needed.

4. The flow rate of carrier and reagent streams can be reproducibly controlled including predefined intermittent stop and run periods.

5. Improved separation steps can be incorporated.

The question about the comparison between robots and FIA is not as difficult as some would like us to believe. The robotic systems, although clever in their application, do have some limitations. The robot requires extremely precise handling of its environment (i.e., the beakers, flasks, and bottles all have to be in the same place at the right time day in and day out). The robot also places a most interesting set of restrictions on the analyst. These include geometry and space requirements, large time investment for software development and parallel operations. With time technology will most likely overcome these problems, but for now the FIA system is just as automated and less finicky about its daily work environment. The robotic systems definitely have a place in extremely dangerous situations (i.e., radioactive assays or highly toxic materials). However, with respect to sample throughput, an FIA system combined with an intelligent automated sample carousel is just as, if not more effective, in the analytical laboratory. The high sample throughput resulting from the elimination of the air bubble makes FIA a reliable and cost effective approach for automation of analytical methods. Additionally, the use of separation and matrix modification techniques are easy to incorporate thereby improving method selectivity.

1.4   OBJECTIVES OF THIS BOOK

The objectives of this book are simple:

1.   To provide the reader with a practical guide to FIA.

2.   To provide examples of currently used and clever applications of FIA in analytical and process control chemistry.

3.   To provide a selective bibliography that gives the reader a ready to use catalog of the FIA literature.

At no time was the purpose of this work to describe and derive the theories behind the FIA technique. It is more important to the potential users to understand the correct procedures, the problems and the uses of FIA. Flow Injection Analysis is a tool to do chemistry. That means: the analytical chemists of the world had better brush up on their chemistry.

REFERENCE

1.   L. Skeggs, Am. J. Clin. Path., 28 (1957) 311-322. [-]

CHAPTER 2

FIA PRINCIPLES AND THEORIES

2.1   INTRODUCTION

This chapter will describe the principles and theories of FIA. A large number of theoretical equations will not be used as the goal of this book is to explain the basic principles and how they will or will not affect the FIA system being designed. It is more important for an FIA user to develop common sense or intuition about the technique than to study the rigorous mathematical treatment of theories that may or may not be correct at this time. Therefore, before a discussion of FIA principles can be started it is important to survey the reasons for the development of FIA. To do this, an examination of the basic principles of batch methods, segmented continuous flow analyzers, discrete analyzers and the concept of the steady state must be critically performed. Only then will the development and principles of FIA seem straightforward.

2.2   BATCH METHODS

Let us examine batch methodology. To start, there is nothing wrong with batch chemistry methods. In fact, many of the current automated methods are based on the batch method predecessor. Batch methods were designed to maximize precision and accuracy and few had to be used in situations where large numbers of determinations per day were needed. These methods require highly skilled chemists to carry out the individual tasks.

Fig. 2.1 depicts a typical batch process. A known amount of sample (S) and analytical reagent (R) are brought together into a common beaker. This actual physical pouring or addition by pipeting is not critical to the reproducibility of the chemical system. However, the establishment of the chemical equilibrium

$$R + S \rightleftharpoons RS$$
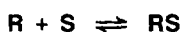
has two major components, physical mixing and chemical kinetic rates.
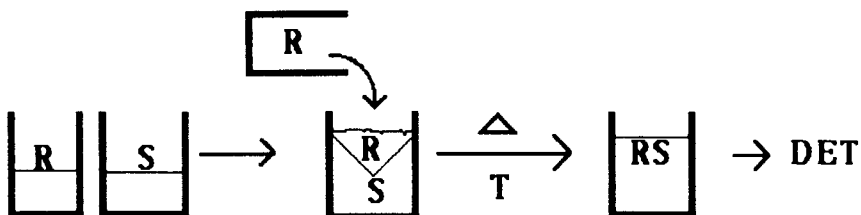
Fig. 2.1. Batch process where R is the analytical reagent(s), S is the sample, RS is the reaction product that is measured, T is time, △ is heat and DET is any detector.

Investigation of physical mixing reveals that it is accomplished by convection and diffusion. Convection results when R and S are mixed by agitation. In other words, this procedure is superimposed on the system and its magnitude can easily be controlled. Diffusion, on the other hand, is an inherent process over which the analyst normally has little control. The convection caused by the agitation is actually random turbulence. In the case of batch methods the establishment of the physical mixing equilibrium is extremely dependent on the rate of agitation. Therefore, a reproducible signal created by the RS complex at a given time is dependent on a reproducible rate of mixing.

The chemical kinetic rates are dependent on the reagent and sample concentrations which, are primarily a function of physical mixing at any of the interfaces between R and S, and also the initial concentrations, temperature and pressure and the kinetic order of the reaction(s). For batch methods, pressure is constant and temperature is controlled. Unless the sample concentrations are extremely high or low the kinetic order does not change. The kinetics are usually believed to be understood or at least consistent enough to establish reproducible and predictable results. The variability in the attainment of chemical equilibrium in the batch method is primarily dependent on the rate of mixing. Reproducibility of the signal for the RS complex at a given time is therefore dependent on the rate of mixing and the consistency of pressure, temperature and kinetic reaction rates.
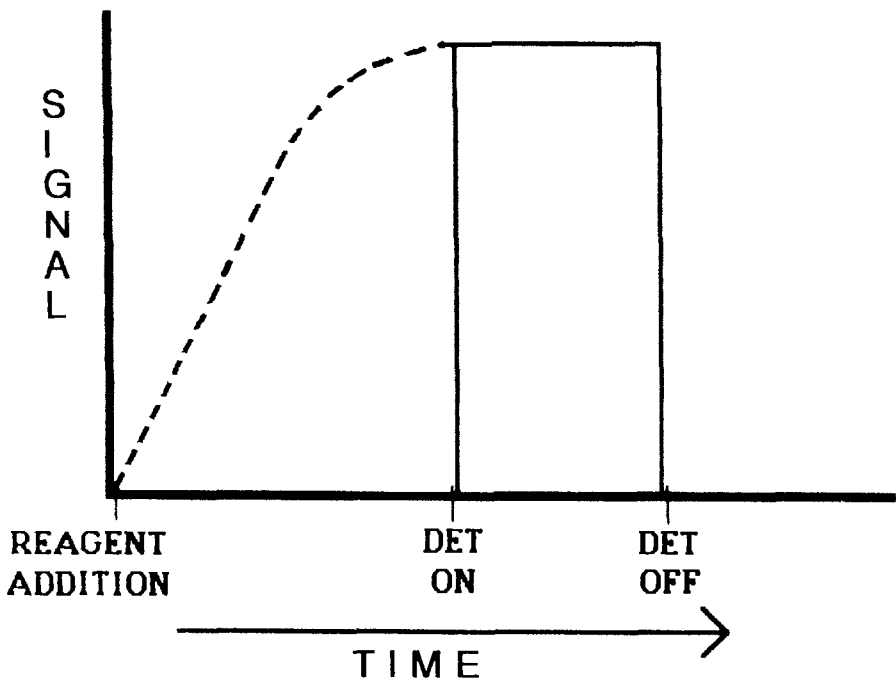
Fig. 2.2. Typical batch method signal plot where DET ON denotes the time at which the insertion of the sample into the detector takes place and DET OFF corresponds to the removal of the sample from the detector. The broken line is an estimate of the signal response and the solid line is the observed response.

Fig. 2.2 is a plot of the observed signal for a batch method. The plot of signal versus time is represented by a broken and then a solid line. The broken line is the best estimate of the signal that would be observed if it could be measured. In batch methods the agitation of the sample makes the actual measurement of the points on the broken section of the plot impossible. Even if the sample could be measured, the irreproducible physical mixing would make the reproducibility of the signal unacceptable. The solid line is a computation of the average of the points that are observed.

The plots in Fig. 2.3 demonstrate the difficulty encountered when trying to measure at a point other than steady state when using a batch method.
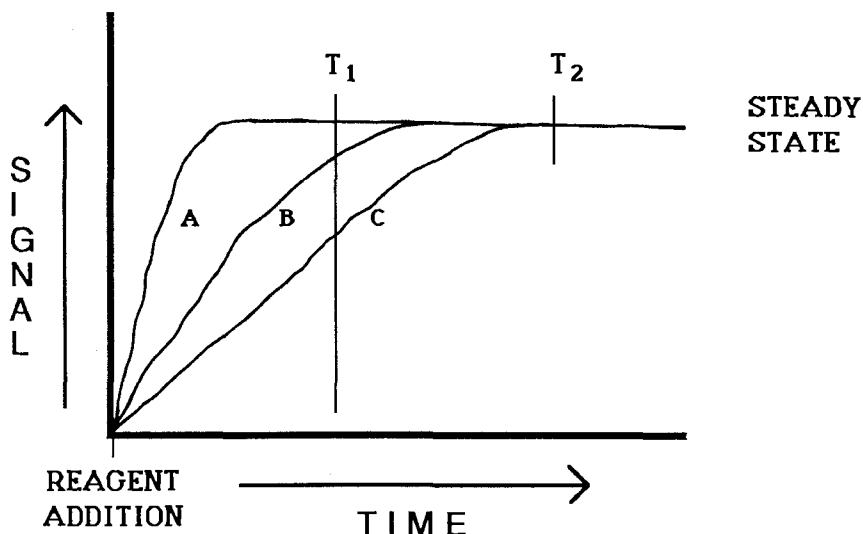
Fig. 2.3. Batch method signal versus time plot. Runs with three different samples of the same concentration.

At time T_1 three distinctively different values for the same sample are obtained. Only at time T_2 are the values similar within experimental errors. Unfortunately, for the batch method only steady state signals can be used to obtain reproducible measurements.

2.3   SEGMENTED CONTINUOUS FLOW ANALYSIS

Eventually, the demand for a large number of analytical determinations per laboratory became apparent. The obvious answer was to automate the batch method approach. The basic concept was to reproduce the beakers of the batch method inside a flowing stream. The resulting technique was the air-segmented continuous flow analyzer. Fig. 2.4 shows the classical single reagent manifold for air-segmented continuous flow analysis. Both R and S are fed by a peristaltic pump into the tubing where they eventually react. Once again, this rate of reaction is dependent on physical mixing and chemical kinetic rates.

AIR

AIR

S

R

DET

MIXING TUBE

PUMP
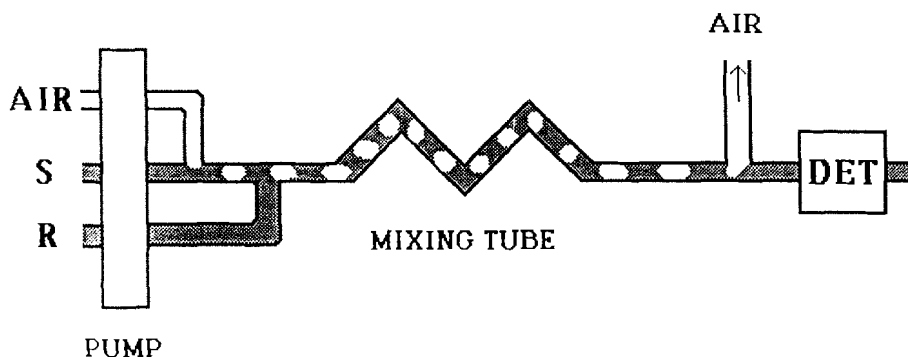
Fig. 2.4. Schematic diagram of the classical air-segmented continuous flow analyzer.
R = reagent, S = sample, DET = flow through detector.

As the air-segmented continuous flow analyzer was originally designed it represented nothing more than automation of the batch method. This is true because proportional mixing is still involved. In a batch method proportional mixing is obtained when a known volume and concentration of R is poured into a known volume of S. In air-segmented continuous flow analyzers proportional mixing is obtained similarly when a constant flow of R is fed into a constant flow of S. In other words, R is merged with S at a specified, constant, and controlled ratio.

The air segments actually create the walls of the "beakers". As the "beakers" travel down the tubing, the solution drags against the walls of the tubing, thus creating the necessary mixing and convection (Fig. 2.5). The important point about segmented continuous flow analyzers versus FIA is that the air bubbles are used to limit the amount of dispersion that can occur. Molecular diffusion is a minor component in the overall mixing process.

FLOW

S   A   S   A   S   A
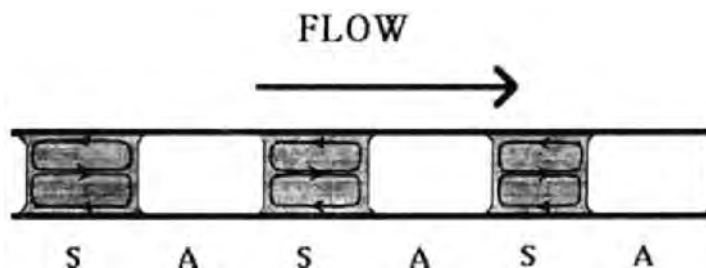
Fig. 2.5. Diagram showing the mixing pattern inside a liquid segment due to drag on a glass wall. Arrows denote the direction of the convection processes.
A = air segments, S = sample segments.

The question is: what should the detector response ideally look like for an air-segmented continuous flow analyzer? Fig. 2.6 shows the ideal plot of signal versus time.
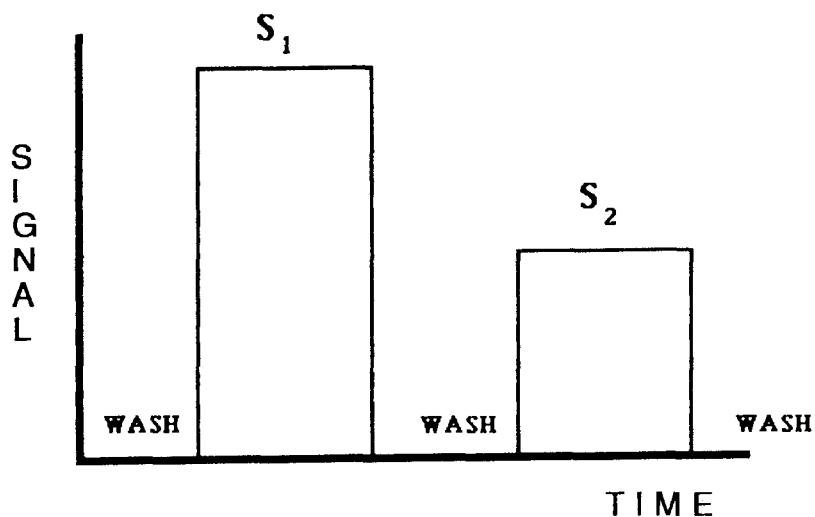
Fig. 2.6. Ideal output from air segmented continuous flow analyzer.
Sampling strategy: wash, S_1, wash, S_2, wash, where $S_1 = 2S_2$ in concentration.

However, in practice the air-segmented continuous flow analyzers produce signals such as those shown in Fig. 2.7.
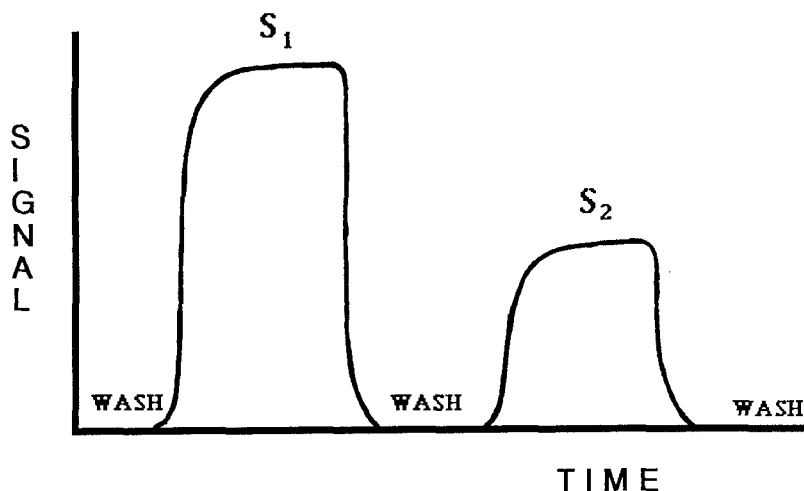
Fig. 2.7. Actually signals obtained from an air-segmented continuous flow analyzer.

Why does this skewing of the actual air-segmented continuous flow analyzer peaks appear? The origin of this phenomenon is carryover. Carryover occurs when R or S molecules remain on the walls of the tubing and pass on to the following segment. The liquid film that forms on the tubing walls makes the carryover process most influential on the physical equilibrium. Therefore, the rising portion of the output is a gradual increase to a "steady state" signal and the falling portion of the output is a gradual return to the established baseline.

For classical air-segmented continuous flow analyzers the only portion of the peak that the analyst can use for quantitation is the steady state plateau. The compressibility of the air bubbles affects mixing, i.e., the physical equilibrium, which in turn is the controlling factor on the rate of acquisition of the steady state signal.

The batch method and classical air-segmented continuous flow analyzers handle the physical and chemical equilibrium processes in a similar manner. Consequently, they both require that steady state signals are used to obtain reproducible results.

In fairness to air-segmented continuous flow analyzers, these instruments do automate the tedious laboratory manipulations and increase the precision of results by eliminating human factors. These systems are capable of multichannel (more than one analyte determination per sample introduction) and multitask (dialysis, heating, extraction, etc.) analysis. More recent refinements of this technique, such as miniaturization and gated detectors, make the removal of the air bubble unnecessary. Additionally, computer predictions of the steady state signal can be made by observing the initial portion of the rising section of the output, thereby increasing the sample throughput.

2.4   DISCRETE ANALYZERS

A discrete analyzer handles each individual sample as a separate entity and is usually a single channel instrument. In some designs both the sample and the analytical reagents are metered into discrete reaction vessels. However, most of these systems have specially designed cells that already contain prepacked amounts of the required reagents for the given analyte, thus making sample introduction the only necessary step. In both instances, all the processes in the method (mixing, heating and the determination itself) take place inside the cell or packet. These cells are placed on a moving path (conveyor belt or turntable) and the reacting species are mixed.

A computer can be used to monitor and control the analyzer and also to collect and manipulate the analytical data. Diagnostic software for identifying system malfunctions is usually included. There is some flexibility in the mixing times and detector parameters which allows for some optimization of the analytical method.

Basically, discrete analyzers allow established manual techniques to be directly emulated. This means, of course, that steady state signals are necessary. Even though the mechanical engineering of these systems is a marvel to behold, it is at the same time the limitation of the system. The mechanical complexity and cost are significantly greater than those of the air-segmented continuous flow analyzer or the flow injection analyzer.

Given the current state of FIA, the only real advantage of discrete analyzers is the availability of prepacked reagents. Together with computer control, this makes for nearly "foolproof" operation. However, it is assumed that the same determinations are run day in, day out without variation in the sample matrix or other complicating factors. Many laboratories need a flexible system which can be modified to meet the analytical needs created by variations in sample composition. The rigid design of discrete analyzers is a major limitation as it minimizes this flexibility. An additional limitation is that the cost of the discrete analyzers is much higher than that of any of the continuous flow analyzers.

2.5   FLOW INJECTION ANALYSIS

The automation of chemical methods is necessary for cost, safety and reliability reasons and has been realized through the development of air-segmented continuous flow analyzers. Since careful control of the air bubbles is critical to the optimized performance of these systems, start up and stabilization require considerable time. Is there a better way to do automated continuous flow analysis? Yes!

The question that must be asked is, what is needed to produce an automated system with high reproducibility? It has been shown in the previous sections that the answer is a system in which the physical mixing can be carried out reproducibly. Neither batch methods nor air-segmented continuous flow analyzers have reproducible physical mixing. The air bubbles in the air-segmented continuous flow analyzers are the major source of randomness in the system. They cause irreproducible mixing and therefore create the need for a "steady state" measurement. Why use an air bubble? The purpose of the air bubble is to create the "beaker" walls. The bubbles are placed in the system to prevent intermixing of segments or samples. What happens when the bubbles are removed from the system? The answer is Flow Injection Analysis (FIA)! As a consequence of the air bubbles being removed from the system, the physical mixing of the reagent and sample can be reproducibly performed.

As shown in Fig. 2.8, in FIA the sample is injected into a nonreactive carrier stream, not introduced into the system through the pump. In practice, this carrier stream may be a pH or ionic strength buffer or simply water. The sample plug is pushed down the tube to the tubing tee or junction. The reagent is pumped down another tube towards the

same tee. The reagent is usually continuously pumped. The carrier stream containing the sample zone is merged with reagent at the tee.

S
↓

CARRIER

REAGENT

MIXING

COIL

DET → WASTE
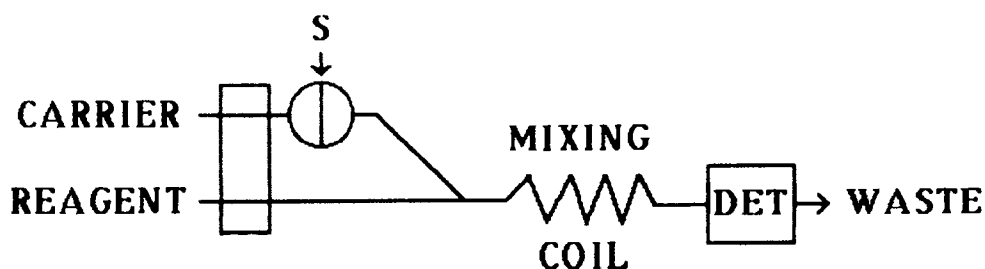
Fig. 2.8. Schematic diagram of a typical single reagent FIA system. S = site of sample injection, DET = detector.

The exact mechanism of how R and S merge is important but the details are beyond the scope of this discussion. However, it is important to realize that the flow characteristics and mixing at this union are very reproducible.

The combined R and S pass through a coiled section of tubing in order to provide the necessary conditions for the desired chemistry. The coil dimensions, the sample volume injected and the flow rates of R and C are optimized for the given chemistry being utilized. But what is meant by optimization? There is no straightforward answer to this question because each method should be developed for a critical need, i.e., high throughput, good selectivity, low detection level or specific detector requirements. The optimization is used only to achieve the desired characteristics for the method.

The FIA system itself provides the reproducible physical conditions, in contrast to batch methods or air-segmented continuous flow analyzers. This is true since the FIA system contains no components that create random turbulence. Only reproducible convection will be observed. The next question is whether a steady state signal is a requirement for precise quantitation in an FIA system. Since identical physical and chemical conditions can consistently be obtained, as long as the system configuration is not changed, steady state is not a necessary requirement.
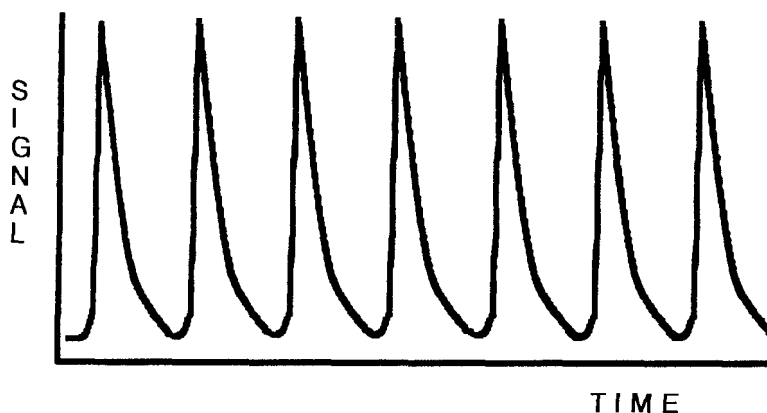
Fig. 2.9. Typical detector output of an FIA system into which a dye solution is repeatedly injected.

Fig. 2.9 is a printout of multiple injections of a dye and is a typical example of the physical reproducibility of FIA.

The next questions are, does the addition of the chemistry to the system affect this reproducibility and is it necessary for a chemical reaction to reach completion before detection?

Fig. 2.10 shows the development of an analytical signal with respect to time where the reaction is eventually complete (point B).
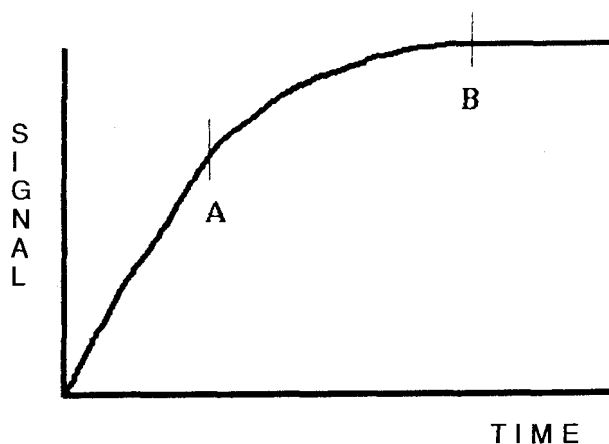
Fig. 2.10. Development of an analytical signal with time.

It is clear that point A could not be reproducibly measured in the batch and air-segmented continuous flow systems. However, the reproducible timing and the subsequent reproducible physical mixing make the measurement of point A using FIA highly precise. The addition of chemistry to the system does not appear to significantly alter any of these physical characteristics. Although the chemistry (kinetics) is also affected by temperature and pressure, these can be kept constant in the FIA system.

2.6   CONTROLLABLE SAMPLE DISPERSION

The three basics of FIA, as defined by Ruzicka and Hansen, are **reproducible timing, sample injection** and **controlled dispersion** (1). As the FIA technique has been developed further, these three basic principles have been modified. For example, samples are not always injected using a valve. Sample introduction is accomplished as long as a sample plug is reproducibly inserted into the flowing carrier stream.   Without question reproducible timing is the critical principle, since it leads to the reproducible physical conditions. The term "controlled dispersion" has created much confusion to the scientific community. In this section, controlled dispersion, as it was originally explained, will be described. Then controllable sample dispersion will be discussed.

Once the reproducible timing of the FIA system had been realized, it was observed that the injected sample zone always seemed to spread out inside the manifold in a reproducible manner. By observing the peak shapes and heights it was clear that the sample zone reproducibly expanded or diluted in the flowing stream. In order to quantitate this phenomenon, Ruzicka and Hansen described a series of experiments that would measure this expansion. They called the process **dispersion.**

Dispersion was then defined as the amount that the chemical signal is reduced by injecting a sample plug into an FIA system. This is represented mathematically by

$$D = C^{\circ} / C^{max} \tag{2.1}$$

where D is the dispersion coefficient at the peak maximum produced by the ratio between C°, the concentration of a pure dye, and C^{max}, the concentration of that same injected dye as it passes through the detector.

The actual experiment to measure D is as follows. A dye such as bromothymol blue is pumped through the entire FIA manifold, i.e., both the carrier and the reagent lines. The signal is evaluated and the concentration, C°, which is representative of the pure, undiluted dye solution, is measured. Then, the dye is replaced with a carrier solution which is pumped through the system. With bromothymol blue borax is used in order to maintain the proper pH. Finally, the bromothymol blue solution is injected into the

manifold. The observed signal is recorded and C^{max} is calculated. The dispersion coefficient can then be calculated.

The dispersion coefficient is useful in that it allows comparisons of different manifolds. Further, it provides a means of verifying and monitoring the extent of sample dilution resulting from any changes made to the manifold during method development. In essence, what Ruzicka and Hansen called controlled dispersion is in fact the recognition that the sample is reproducibly diluted as it travels down the tubing. The reproducible timing allows for reproducible physical mixing and dilution. The dispersion is controlled. But there is a second aspect to the controlled dispersion feature.

It is clear that the analyst has complete control over the extent of sample dispersion or dilution that occurs as the sample passes through the manifold. This control comes from the way in which the manifold is designed. Different degrees of sample dispersion are necessary depending on the application being carried out or the detector that is used.   For example, the dispersion coefficient can be less than 1 for preconcentration, between 1 and 3 for selective detectors, between 3 and 10 for chemical manifolds and some separation techniques, and greater than 10 for FIA titration. What should actually be said is that FIA has **controllable sample dispersion**.

2.7   FACTORS AFFECTING CONTROLLABLE SAMPLE DISPERSION

In practice, the analyst controls the amount of sample dispersion by altering the manifold design.   This controllable sample dispersion allows for a large degree of flexibility for the analyst. Preconcentration all the way to dilution can be carried out by using different FIA manifold designs. The design of the manifold is dependent on the chemistry/detection system that is chosen by the analyst. Therefore, the amount of sample dispersion is dictated to the analyst by the chemical method that has been chosen. What factors influence the sample dispersion in an FIA system? To simplify the thinking, let us use the bromothymol blue dispersion manifold shown in Fig. 2.11.
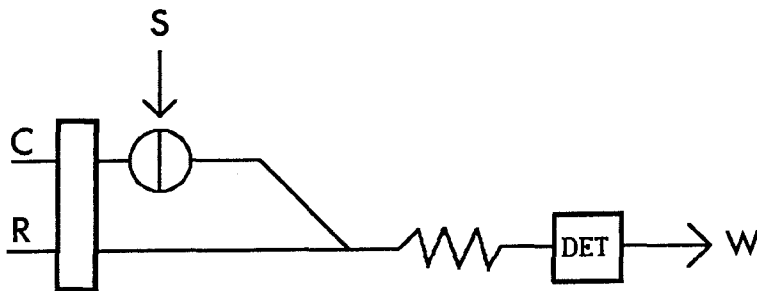
Fig. 2.11. Schematic diagram of the manifold used for dispersion experiments.
C = carrier, R = reagent, S = sample, DET = detector, W = waste.

Note that no chemical reaction is involved in this experiment. Perhaps the factor that first comes to mind is the total flow rate, i.e., varying the pump speed without altering the manifold design.

Table 2.1 shows some typical results of such experiments. It can be seen from the Table that total flow rate has a very small influence on sample dispersion. But what about differential flow rate variations between the carrier, C, and reagent, R?

TABLE 2.1

Dispersion coefficient, D, as a function of the total flow rate for a fixed manifold design. Sample volume: 100 μl. The flow rates of the carrier and reagent are identical.

Total flow rate ml/min	Dispersion coefficient D
1.6	3.0
2.4	2.9
4.0	2.9

Table 2.2 gives the answer: differential flow rate variations produce significant changes in sample dispersion.

TABLE 2.2

Dispersion coefficient, D, as a function of the C/R ratio. A fixed manifold with a sample volume of 100 μl.

Flow rate ml/min		C/R	C/(C+R)	Dispersion coefficient
C	R			D
2.0	2.0	1.00	0.50	3.0
2.0	1.2	1.67	0.62	2.4
1.2	2.0	0.60	0.38	4.0
0.6	2.0	0.30	0.23	6.5
2.0	0.6	3.33	0.77	2.3
2.8	0.4	7.00	0.88	2.0
0.4	2.8	0.14	0.13	11.7

The explanation is straightforward. At the merging point of C and R different degrees of dilution occur. For example, in the case of a C/R ratio of 7 we have, at the merging point, seven volumes C (and S) for each volume of R. Although this appears to be an effective way of controlling sample dispersion, there seems to be a practical limitation to maintaining constant flow rates at ratios smaller than 0.1 and larger than 10.

The next suggestion would be to change coil length in Fig. 2.11 in order to change the sample dispersion. Table 2.3 shows the dispersion coefficient, D, as a function of coil length.

TABLE 2.3

Dispersion coefficient, D, as a function of the coil length. A fixed manifold with a sample volume of 100 μl.

Coil length, cm (i.d.=0.5 mm)	Dispersion coefficient D
30	3.0
60	3.0
120	3.3

Clearly, coil length does not significantly affect the sample dispersion. Would this observation still be true for straight tubes of similar lengths and diameter? The answer is that the relative amount of sample dispersion is slightly greater in the straight tube. This is expected in view of fluid dynamic theory. What happens when the inner diameter of the coil or tube is changed?

Table 2.4 shows dispersion coefficient data for different inner diameters. The conclusion is that the inner diameter, within practical limits (0.35 - 0.9 mm), is not critical to sample dispersion.

The overall conclusion about coil/tube length and inner diameter of the coil is that, relative to flow rate ratio, they are minor contributors to changes in sample dispersion. Therefore, utilization of these factors for manipulation of sample dispersion is not recommended.

TABLE 2.4

Dispersion coefficient, D, as a function of the coil inner diameter. A fixed manifold with a sample volume of 100 μl.

Coil i.d., mm (length 60 cm)	Dispersion coefficient D
0.35	3.0
0.5	3.1
0.7	3.2
0.9	3.8

The last choice is to vary the sample volume. Will such a change produce significant changes in the observed dispersion coefficient? Table 2.5 shows sample dispersion coefficients as a function of sample volume. As can be seen, a threefold decrease in sample dispersion occurs when the sample volume is increased from 40 to 400 μl. The ease with which sample volume can be changed makes this approach one of the most convenient ways to manipulate sample dispersion. However, a practical restriction is that it cannot be changed more than a factor of 3 - 4.

TABLE 2.5

The dispersion coefficient D as a function of the sample injection volume.

Sample volume μl	Dispersion coefficient D
40	6.1
100	2.9
200	2.2
400	2.1

The above discussion is based on the manifold configuration shown in Fig. 2.11. By changing flow rate ratios, sample volumes, coil length and diameter in this manifold a tenfold variation of sample dispersion can be achieved. Obviously, there are situations

where greater than tenfold dilution is required. Consequently, the manifold must be redesigned to fulfill this requirement.

A mixing chamber can be incorporated in the manifold as depicted in Fig. 2.12. The mixing chambers can be of two types: passive or active.
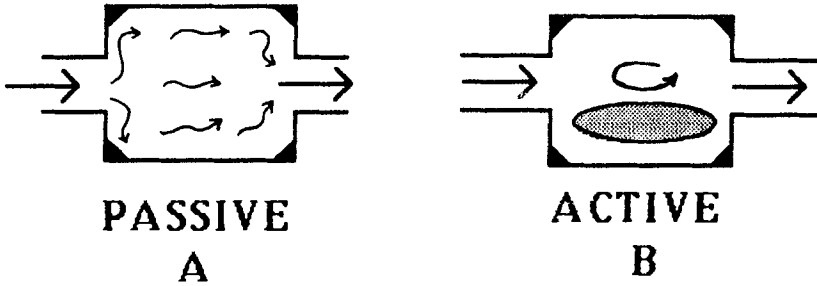
PASSIVE
A

ACTIVE
B

Fig. 2.12. FIA systems with a mixing chamber included to promote dilution of the sample: (A) passive and (B) active mixing chambers.

Regardless of the type of mixing chamber the effect of such devices is presented in Fig. 2.13.
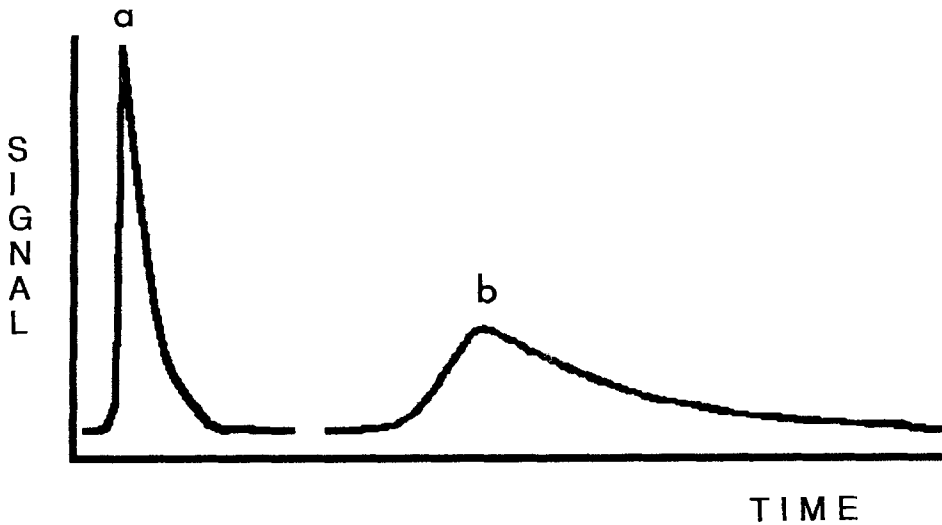
Fig. 2.13. FIA peak without (a) mixing chamber and with (b) mixing chamber.

The peaks contain the same amount of dye, but the peak shapes are markedly different. The mixing chamber creates a broad peak with a large tail. Obviously, mixing chambers will reduce the sample throughput of an FIA system, as the introduction of the next sample normally would not be made until the entire tail of the preceding sample has left the detector.

In an effort to avoid the broadening of the peak and the tailing created by the mixing chamber, a variety of techniques have been developed. Zone sampling is based on injection of the sample into a carrier where it is diluted, thus creating substantial peak broadening. The dispersed sample is then led into a second injector which cuts out only a portion of the entire sample zone and injects it into a second carrier, see Fig. 2.14. The timing between injections 1 and 2 is critical with respect to reproducibility and can be used to vary the degree of dilution. Dispersion coefficients of up to 1000 can be achieved with this technique.
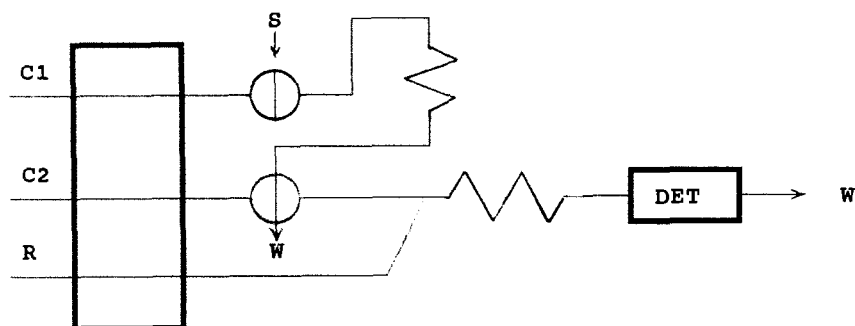
Fig. 2.14. Manifold design for zone sampling. C1 and C2 = carrier streams, R = reagent, S = sample, DET = detector, W = waste.

Sample splitting can be performed in several ways. Fig. 2.15 shows a simple manifold for sample splitting.
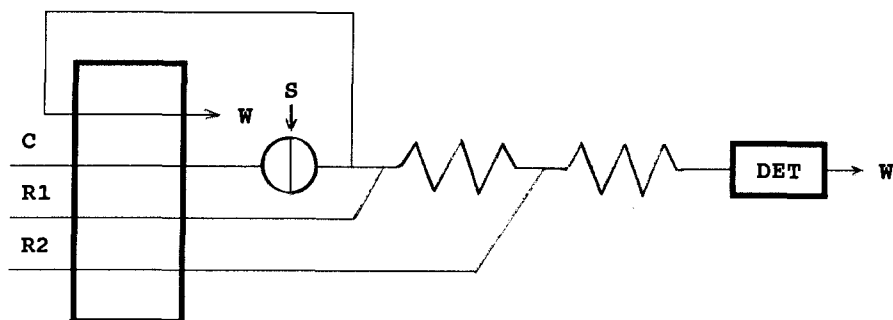
Fig. 2.15. Manifold design for sample splitting. C = carrier stream,
R1 and R2 = reagents, S = sample, DET = detector, W = waste.

Immediately after the injection of the sample zone a major portion of the carrier stream is diverted to waste and replaced with a new carrier (or reagent) stream. Note that the distance between the diversion point and the merging point must be kept short because the flow rate is low between these points.

Once again, it must be stressed that the discussion in this section has not involved any chemical reactions.

2.8 RELATIONSHIP BETWEEN MANIFOLD DESIGN AND CHEMISTRY

In the previous section, the discussion centered on the effect that physical changes in the FIA system have on dispersion of the sample. It was clearly demonstrated that controllable sample dispersion is possible with FIA. However, in that section the effect that these manifold design changes have on the chemistry, and therefore the observed signal, was not discussed. Usually, changes in manifold design are made in order to meet the specific needs of the particular method/detector that is being used. The optimization of the design needed for the chemistry usually takes precedence over the concern for the minimum amount of sample dispersion. So, let us ask, what changes in an FIA system would affect the chemistry as reflected by the observed signal?

Changes in total flow rate could affect the observed signal since the time that the sample and reagent are mixed before detection is changing. This time is called the reaction time. Increases in the total flow rate result in a decrease in reaction time. If the chemical reaction is not at equilibrium, as in many FIA methods, the time available for the

reaction to proceed toward equilibrium is reduced. Therefore, a lower signal is observed because the formation of RS is reduced. Changes in the ratio between flow rates introduce two potential factors into the chemistry. First, the total flow rate could be changed and therefore the reaction time would be changed, as already discussed above. Second, the change in the relative concentrations of R and S may change the apparent rate of RS formation. This is dependent on the kinetics of the chemical reaction and that the reaction has not reached equilibrium.

Changes in coil length would obviously change the observed signal for a chemistry that has not reached equilibrium since an increased coil length increases reaction time. Changes in coil inner diameter may or may not affect the observed chemical signal. If the changes are small such, as 0.3 - 0.5 mm i.d. tubing with total length of less than 1 m, the reaction time is not dramatically increased. Therefore, the change in signal is not significantly different. However, if the changes are large, such as 0.3 - 0.9 mm i.d. tubing or combined with increases in length, the reaction time is significantly increased. Therefore, if the steady state condition has not been reached, the observed signal will be enhanced.

Obviously, changes in sample size will affect the observed signal. However, most of this observed change in signal is due to the physical dispersion characteristics and not to any chemical kinetic effects. The sample size does not alter the sample concentration at the merging point between R and S. It only increases the length of the sample zone.

In conclusion, there are only two ways that the kinetics of a chemical reaction can be used to change the observed FIA signal: change in reaction time and varying the ratio of R and S. Since the chemical kinetics are usually not well understood or the practical limitations of the FIA system do not provide a large enough change in ratios to allow for a significant change in the apparent rate of reaction, the only practical approach is to increase the reaction time. The best way to increase the reaction time is to decrease the flow rate. The next best way is to increase the coil length. Neither of these approaches significantly increases physical dispersion. Any manifold change that increases the reaction time and does not significantly change dispersion is the best way to utilize chemical kinetics.

Two points need to be mentioned when dealing with chemical kinetics. Obviously, temperature should change the rate of reaction. It is possible to have a heated section of manifold to promote a reaction. Second, a stopped flow procedure can be used. Both techniques place special requirements on the FIA hardware; these techniques are discussed later.

2.9   THE GRADIENT

The output of the FIA system, as shown in Fig. 2.9, hides a basic feature of FIA. Fig. 2.16 shows an expanded view of an FIA peak. The peak is skewed. The question to be asked is: why does this skewing exist? The answer is that as the sample zone travels down the manifold dispersion occurs. However, the amount and direction of dispersion are not uniform for the entire sample zone.
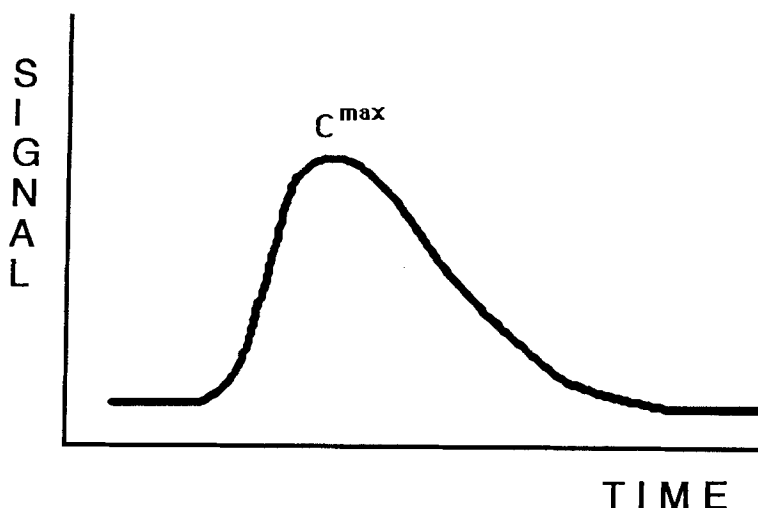
Fig. 2.16. Expanded x axis (time axis) for an FIA peak. C^{max} denotes the maximum analyte concentration.

The center of the zone is moving faster than the edges against the wall, as wall drag slows the molecules. This is called axial dispersion. The molecules against the walls also move back toward the center of the tubing. This is the radial dispersion of the system. Both radial and axial dispersion occur as the zone moves down the tubing. This dispersion process has been described in several ways and many theories about the exact appearance have been presented.

The most common depiction of the dispersed sample zone is shown in Fig. 2.17. The actual length of the plug is considerably more extended than shown in Fig. 2.17. In addition, the parabolic shape of the peak head portion has been questioned. Since the concentration is greater in the center and at the front of the zone, the FIA peak quickly rises to a maximum. The tailing section of the zone creates the slowly decreasing backside of the peak to the baseline.

FLOW →

DISPERSED SAMPLE PLUG
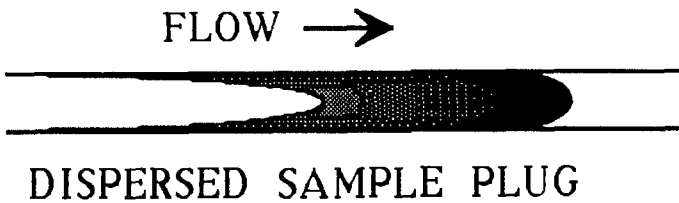
Fig. 2.17. Simplified depiction of the sample zone after some dispersion has occurred.

When comparing the air-segmented continuous flow analyzer peaks with the FIA peaks there are obvious differences, see Fig. 2.18.
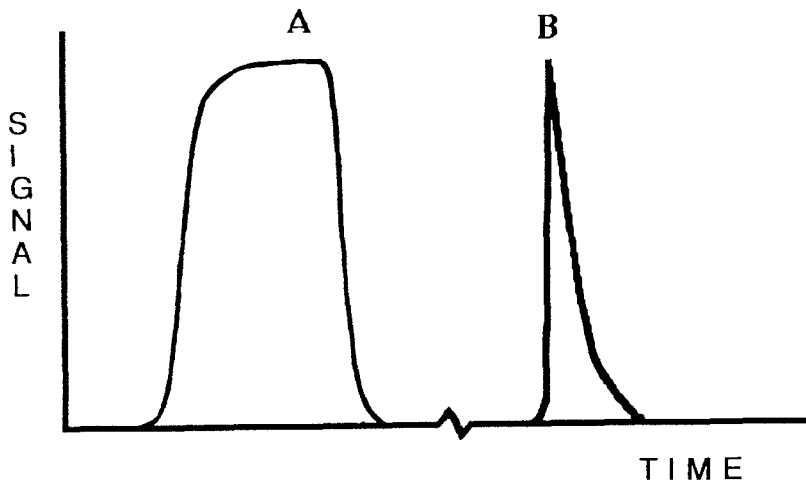
Fig. 2.18. Comparison of signal output from an air-segmented continuous flow analyzer (A) and from a flow injection analyzer (B).

Why do these techniques produce different peak shapes? The answer is that the segmentation produces irreproducibility, thereby requiring steady state measurements. In FIA a steady state is not a necessity. The bubbles and the steady state signal basically lead to the objective of the air-segmented flow analyzer. The objective is to continuously produce a set of conditions where the ratios between R, S and RS are the same at a given time for different readings of the same sample. Once again, the only place where this objective can be met is at the plateau or the steady state portion of the curve. Although it is potentially possible to use the rising portion of the peak to model and predict the steady state signal, it requires a significant assumption. The sample and standards must be matched in terms of matrix, interferences, and other conditions which would affect the way in which the rising portion of the peak is represented. Therefore, either the samples are uniform or the chemistry is unaffected by these changes. Situations where these assumptions are true are rather limited.

But does not FIA have the same objective, namely to continuously produce a set of conditions where the ratios between R, S and RS are the same at a given time for different readings of the same sample? Yes, it has! But for FIA any given time can be used, not necessarily the time at which the maximum signal is attained. As a result, several sets of analytically useful ratios for R, S and RS are obtained, rather than just a single set. In fact, any point on the ascending part of the curve has a corresponding point on the descending part at which identical conditions for the concentration ratios are valid. The reproducible spectrum of different concentration ratios is unique to FIA and is called "the gradient".

Let us explore this concept further. Assuming that the sample is injected into a carrier stream which is merged with the reagent, the amount of RS that is formed depends on the kinetic rate of reaction between R and S and the time of reaction that the sample has in the FIA system. Assuming no chemical reaction, the ratios between R and S would remain constant at a given time for every injection of the same sample and with a constant sample loop size. If the assumption is that 50% reaction has occurred, then the ratios between R, S and RS will be the same at a given time for every injection of the same sample and with a constant sample loop size. If 100% reaction occurs, then the ratio between R, the excess of reagent, and RS at a given point will be reproducible for every injection of the same sample and with a constant sample loop size. In all instances a concentration gradient is formed.

Earlier, the dispersion coefficient at the peak maximum was discussed and defined as

$$D = C^o/C^{max} \tag{2.1}$$

However, essentially every section of the peak can be represented by a dispersion coefficient as defined by

$$D = C^o / C \qquad\qquad (2.2)$$

where C^o is the measured concentration when only dye is in the system and C is the measured concentration of the injected, dispersed dye, $0 < C < C^{max}$. This suggests a unique feature of the FIA peak, that is, for every point on the rising portion of the peak, there is an identical point in terms of ratio between R, S and RS on the falling portion of the peak. The dispersion coefficient is then the same for both points. These points are as reproducible as the peak maximum. This unique characteristic of FIA, that a reproducible concentration gradient exists, requires an entire chapter to examine (see Chapter 7). It is an exciting, but at the same time difficult, aspect of FIA.

At this point the reader has sufficient information to understand the basics of FIA. It is suggested that the dispersion experiments outlined in Appendix A be carried out. These experiments will verify the observations and judgements made in Section 2.7 and give the reader some practical experience with an FIA system. The next chapter, dealing with the components of FIA, may be needed as reference material to assist in the experimental operations.

REFERENCE

1. J. Ruzicka and E.H. Hansen, Anal. Chim. Acta, 99 (1978) 37-76. [18]

CHAPTER 3

COMPONENTS OF FIA

3.1   INTRODUCTION

The basic components, their designs and operation are described in this chapter. A researcher can approach FIA from one of three different directions; as a chromatographer, as a continuous flow user or with no experience at all. The usual practice is to read a book or a few articles and design your own system. The usual outcome is that this homemade system is overdesigned. Once a good commercial system is seen, or even the Lego system of Ruzicka and Hansen (1), it is clear that the home built system has been overdesigned. Another problem is that the connections do not have smooth surfaces in the flow channels. This roughness creates additional turbulence which may not be reproducible. The third mistake is to use the wrong type of pump. It is the objective of this chapter to help the reader avoid these time consuming first steps by providing the necessary guidelines and recommendations to assemble a reproducible, workable system. A careful study of FIA papers as well as pamphlets provided by different FIA manufacturers reveals that the cost of an FIA system may range from 1,000 US dollars up to 40 - 50,000 US dollars or even more. This large price range reflects the fact that different capabilities must be designed into a system depending on its intended use. A homemade, inexpensive flow injection unit for educational purposes and an expensive, microprocessor-controlled flow injection analyzer for process control are both, by definition, FIA systems but otherwise these two systems have very little in common. The detector can be a couple of platinum wires but it can also be an atomic absorption spectrophotometer or inductively coupled plasma optical emission spectrophotometer with the resulting price differential being rather significant. Since so many detection principles can be applied in FIA a special chapter (Chapter 4) has been devoted to this subject.

All FIA systems can be subdivided into a number of modular units which, in turn, consists of one or several components. The term "component" will be given a broad interpretation in this chapter from plain tubing, injectors and single line manifolds to complex selectivity enhancement manifolds.

Fig. 3.1 depicts a typical FIA system as shown in most scientific publications. The vessels for carrier, reagent and waste storage are almost always omitted. The detector output signal is, in most cases, recorded on a strip chart recorder.
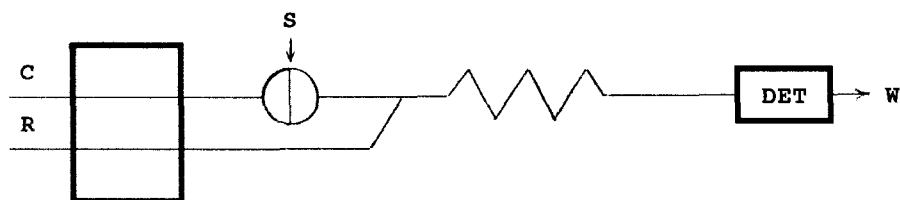
Fig. 3.1. Depiction of a typical FIA system. S = sample, C = carrier, R = reagent, DET = detector, W = waste.

The evaluation of the signal and the storage of data may be computerized but neither the recorder nor the computer is included in the depiction of a typical FIA system. Consequently, a "complete" FIA system should be drawn as shown in Fig. 3.2.
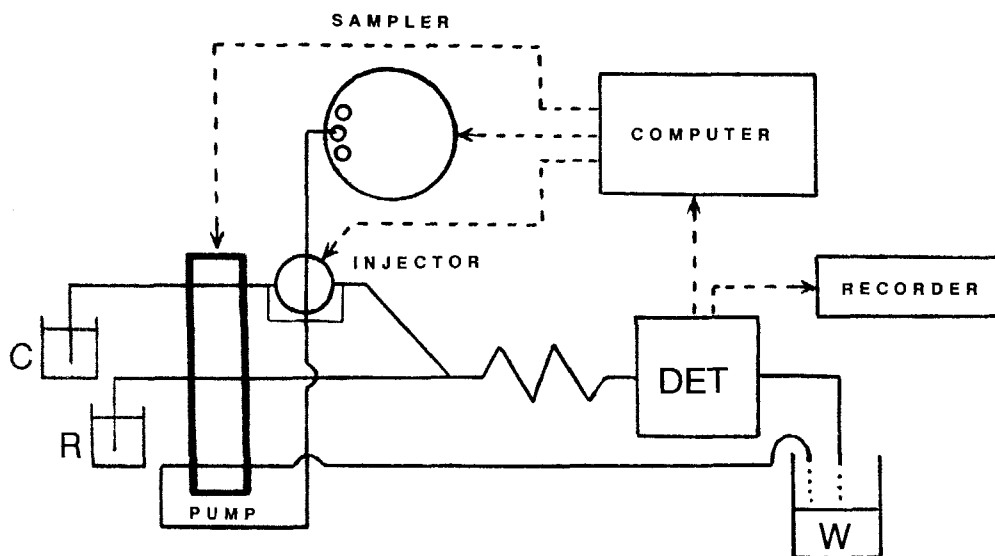
Fig. 3.2. Depiction of an FIA system including all components. C = carrier, R = reagent, DET = detector, W = waste.

The different components in Fig. 3.2 will now be described in detail. Although there are some variations in design which can be tolerated, the common characteristic for all components is that they must do their tasks reproducibly.

3.2 MEANS FOR TREATMENT AND STORAGE OF SOLUTIONS

In Chapter 2 it was stressed that no air should be present in an FIA system. All aqueous solutions contain dissolved gases which may evolve even at room temperature. Reagent and carrier solutions should, therefore, be degassed before use. If thick-walled glass bottles are used for storage of the solutions, the degassing is easily accomplished by applying vacuum (using water suction or a hand vacuum pump) directly on the bottle. By carefully swirling the bottle during vacuum application, the air bubbles which always accumulate on the glass walls can be removed. Once carefully degassed the solutions need no further degassing with normal storage, assuming that the solutions are consumed within about one week. Freshly distilled water, which is commonly used as carrier, needs no degassing at all. Deionized water, on the other hand, contains large amounts of dissolved gas and must be treated. Filtering of reagent and carrier solutions is recommended as a precautionary measure. The two solution treatment steps can be combined using a filtering and degassing unit, see Fig. 3.3.
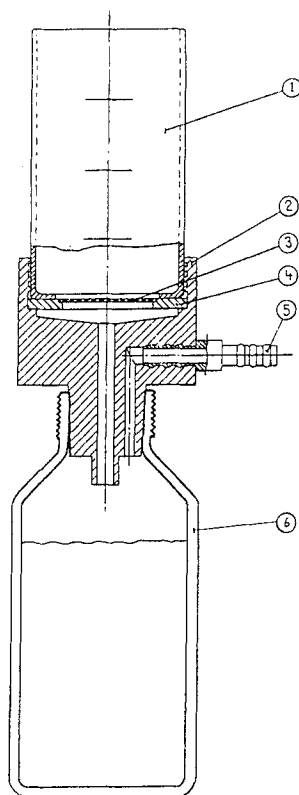
Fig. 3.3. Picture of a filtering and degassing unit. 1. Filling vessel, 2. Vessel holder, 3. Filter and filter grid, 4. Filter grid support, 5. Connector port to vacuum, and 6. Reagent flask.

Since the typical consumption of carrier and reagent solutions is on the order of 1 - 2 ml/min, a vessel volume of 500 - 1000 ml is appropriate. Special caps into which the connection tubes can be fitted will be described in the next section. Amber glass bottles should be used for light-sensitive reagents. Direct sun exposure of all reagents should always be avoided.

3.3 CONNECTION TUBES

Plastic tubes are used to connect the reagent and carrier bottles with the aspiration side of the pump tubes, to connect the press side of the pump tubes with the injector and the manifold, and to connect the detector with the waste container. Tubes of polypropylene, Teflon (PTFE), polyvinyl chloride (PVC) or any other inert material are employed. The most commonly accepted material is PTFE.

It should be mentioned that there is a difference between flow rate and flow velocity. Flow rate can remain constant, but if a larger i.d. tube is connected to a smaller i.d. tube the flow velocity increases. An increased flow velocity may lead to evolvement of gas bubbles since most liquids contain dissolved gas. Therefore, the i.d. of the tubes connecting the reagent and carrier bottles with the pump tubes should be at least 0.8 mm. However, too large a diameter should be avoided since it will then take a long time to start up the system because of the large volume in this area of the system. A hole is drilled in the bottle cap so that the connection tube can be inserted. The tube should be emerged all the way down to the bottom and locked in position using glue or tape. The other end of the connection tube is connected to the pump tube, see Fig. 3.4.
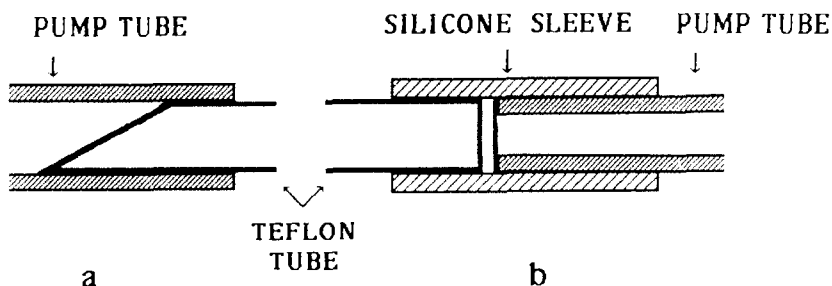
Fig. 3.4. Connection between pump tubes and manifold tubes. For details, see text.

Note that the connection tube has been cut at a 45 degree angle (Fig. 3.4a) to make the insertion easy. The grip on the slippery teflon material is improved if a piece of

sandpaper is folded around the tube. Pump tubes and connection tubes with equally large outer diameters can be connected using a thick-walled silicone rubber sleeve, see Fig. 3.4b. The inner diameter of the connection tubes used in the other parts of the system is not critical, 0.5 - 0.8 mm is appropriate. Be aware of the risk of involuntarily emptying the reagent and carrier bottles due to any siphoning effect.

The waste tube is connected to a waste container with a minimum volume of 2 liter. It can also be connected directly to the sink via a much larger plastic tube (i.d. = 10 mm). If some detergent is added intially to this large tube liquid standstill can be avoided.

3.4   LIQUID DELIVERY UNITS (PUMPS)

The liquid delivery unit is a critical component in an FIA system. There are several technical solutions to the problem of propelling the liquid with a constant flow rate and it is unfair to rate one approach over another. However, since an FIA system is normally operated at pressures lower than 10 psi, there are three types of liquid delivery devices that are utilized; pressurized bottle (including constant head), peristaltic pump, and syringe pump. By far the peristaltic pump has gained the largest popularity and acceptance. If pressures larger than 10 psi are desired any HPLC pump can be used. Most HPLC pumps are reciprocating, positive displacement pumps or high pressure syringe pumps.

The pressurized bottle works according to a principle that is easily understood. Air is provided to a capped bottle containing the liquid to be propelled. A tube is fitted into the cap, led through the cap and immersed into the liquid in the bottle. The resulting overpressure inside the bottle forces the liquid out this tube. In principle, the constant head technique works similarly. The bottle is placed above all other components in the system and the liquid flows due to gravitational force. The flow rate depends on the magnitude of the overpressure, and on the narrowest constriction in the flowpath. It can thus be varied within certain limits. The flow is constant and pulse-free as long as nothing is changed in the system. However, when the sample is injected into the carrier stream the hydrodynamic resistance is changed. Consequently, the carrier flow rate will change. If several pressurized bottles are used in an FIA system, for example one carrier bottle and one reagent bottle, the proportions of the delivered volumes per unit time will be altered as the resistance in one of the streams is changed. This is a definite disadvantage. Another disadvantage is that liquid cannot be aspirated which is desirable for many FIA applications. The main merits of the pressurized bottle technique are that it is inexpensive and simple to apply.

The peristaltic pump consists of a motor-driven wheel with peripherally placed rollers and a compression cam (or band) which is squeezed against the rollers. One or

several pump tubes are affixed so that they rest on a minimum of two of the rollers at all times, see Fig. 3.5.
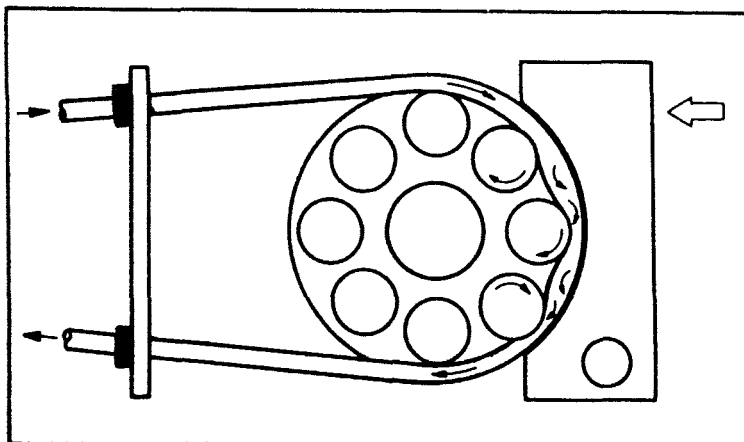
Fig. 3.5. Relationship between the rollers of a peristaltic pump and the pump tubes.

The wheel should rotate at a rate of at least 30 r.p.m. in order to produce a smooth flow. The flow from a peristaltic pump is never completely pulse-free. There is a wide variety of peristaltic pumps and a large number of manufacturers. Some basic requirements should be applied to a peristaltic pump used for FIA purposes. First, the absolute minimum number of channels, i.e. the number of pump tubes that the pump can accomodate simultaneously, should be three. Second, the cam or band tension must be adjustable. Third, the flow rate should be in the range 0.3 - 5.0 ml/min for each channel. Fourth, the pump must be able to start and stop instantly since no extended acceleration or retardation is tolerated for stopped-flow applications.

Other useful properties of a peristaltic pump are: programmable starting and stopping, variable pump speed, reversed rotation, and high-speed rotation for filling, washing and emptying of the flow paths.

The flow rate is varied in two ways, either by changing the diameter of the pump tubes while keeping the roller rotation speed constant, or by changing the roller rotation speed. As mentioned above, there is a lower limit to the roller rotation speed below which the flow can become unacceptable with respect to pulsation. Silicone oil is frequently used to lubricate all the surfaces which are in contact with the pump tubes. This lubrication fulfills several purposes. The life time of the pump tubes is significantly extended. Pulsation is decreased and friction created heat is reduced.

At start-up of a peristaltic pump the lubricated pump tubes are inserted in their respective slots and the compression cam or band is closed. At this time, a minimum of

tension should be applied on the pump tubes. The aspiration side of the pump tube (or its connection tube) is immersed in distilled water. The pump is started and the tension is gradually increased until the liquid just starts to move inside the tube. The tension is then increased a little further. The flow should now be relatively pulse-free. This can be checked by picking the pump tube end out of the liquid repeatedly so that an air-segmented stream results. Such a stream is much easier to study with respect to pulsation. If the pump has separate cams for each pump tube the tension adjustment must be made for each individual channel. If the pump has one common cam or band for all pump tubes it is important that the adjacent pump tubes are fairly equal in size so that the total tension is evenly distributed. The order in which the tubes are placed in the pump could, as a suggestion, be governed by the pump tube size. Each time the flow system is changed during operation of an FIA system, it is advisable to readjust the pump tube tension.

There is no universal method to reduce the pulsation in a system containing a peristaltic pump. Lubrication and a correct adjustment of the tube tension are the basic means to prevent pulsation. Large volume, air-filled pulse-dampeners cannot be recommended. In some cases a piece of a narrow bore tube (= 0.3 mm i.d.) can be implemented after the pump. In other situations the pump tube on the press side can be shortened to prevent "the bellows effect" which may appear for large bore pump tubes.

HPLC pumps are expensive but can in some cases be adapted for use in FIA systems. A depulsing arrangement for positive displacement pumps has been suggested (2). If several pumps are used in the same system the pressure spikes produced by each pump will disturb the proportional mixing of carrier and reagent streams. The flow from each pump can then be restricted with pressure gauges and a needle valve, see Fig. 3.6.
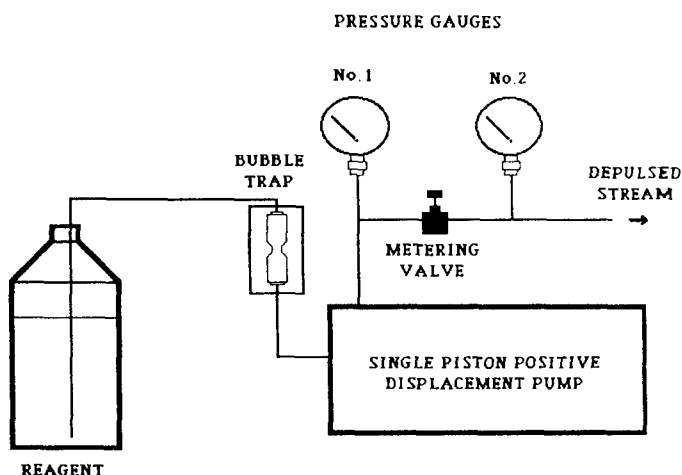
Fig. 3.6. Depulsing system for positive displacement (HPLC) pumps.

The **syringe pump** has not gained widespread use in FIA yet, probably for cost reasons. The most ideal type of this liquid delivery device seems to be the precision piston burette used for titrations. Conceptually, a syringe pump should be able to deliver a pulse-free flow which is insensitive to changes in counterpressure. This has many advantages particularly for applications based on time measurement, for example so called FIA titrations (see Chapter 7.2). Very little is known about the extent of mixing between two streams in an FIA system when pulsations are present. Maybe more attention must be paid to the mixing process when syringe pumps are used.

Organic solvents immiscible with water are propelled easily using the displacement bottle technique. A thick-walled glass bottle is filled with the solvent and provided with a tight cap with connection ports, see Fig. 3.7. Water (Aq) is pumped into the bottle thereby forcing out the organic liquid (Org). Depending on the specific gravity of the solvent the water is expanding into the bottle either from above or from below. The reason for not pumping the organic solvent directly is in most cases that the pump tube material will be destroyed by the solvent.
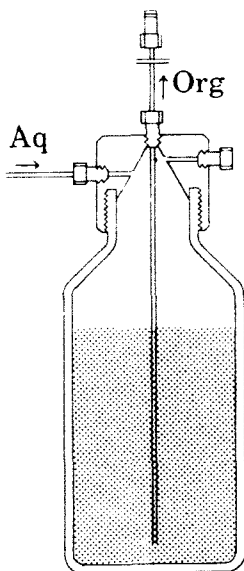
Fig. 3.7. Displacement bottle used to pump organic solvents.

3.5   PUMP TUBES (FOR PERISTALTIC PUMPS)

Pump tubes are made of either PVC, silicone or any other related plastic materials. The most common material is called Tygon, a transparent, PVC-type material. Two collars are usually glued near the end on each pump tube. The purpose of these colored-coded collars is twofold; first, to serve as points of attachment between the harnesses of the pump and, second, to identify the inner diameter and thereby the flow rate. The standard distance between the collars is approximately 15 cm. A much smaller distance, about 6 cm, is used for the Technicon SMA-C pump tubes. Such pump tubes have also been adopted for use in other commercially available pumps. The color code system established for pump tubes is given in Table 3.1.

Tygon pump tubes are used to propel aqueous solutions and alcoholic solutions where the alcohol content should not exceed 5% in order to guarantee a long lifetime. Strongly acidic or basic solutions should not be pumped by Tygon pump tubes. A practical concentration limit is 2 M for both acids and bases.

TABLE 3.1
Color code scheme for some selected pump tubes and corresponding flow rates when used in different pumps.

| Color code | Flow rate, ml/min | | | |
	Autoanalyzer II	Rainin	Tecator 5020 50 Hz 60 Hz	FIAtron*
orange/black	0.015		0.05 0.06	
orange/red	0.03		0.1 0.1	
orange/blue	0.05	0.27	0.2 0.3	
orange/green	0.10	0.40	0.4 0.5	
orange/yellow	0.16	0.70	0.6 0.7	1.7
orange/white	0.23	0.90	0.8 0.9	2.2
black/black	0.32	1.32	1.2 1.4	3.7
orange/orange	0.42		1.5 1.8	4.6
white/white	0.60		2.0 2.4	
red/red	0.80		2.8 3.4	7.4
grey/grey	1.00		3.3 4.0	

* denotes maximum flow rates; pump speed is programmable up to this volume.

Special care must be exercised when an alkaline solution, for example sodium hydroxide, is pumped in a flow system. Alkaline solutions are soapy which may weaken the connections between the pump tube and other tubes. The problem is not only that a simple leakage may occur but that the liquid might easily shower into the eyes of the operator when the connection is breaking. The cornea is very sensitive to alkaline solutions.

Yellow, so called solvent flexible pump tubes (Solvaflex) are used for alcoholic solutions and for some alkanes, for example isooctane. These have collars with the same identification system as described above. Black pump tubes of a viton material (Acidflex) are used for strong acids and for some chlorinated organic solvents, for example chloroform. These pump tubes are relatively expensive and have a much shorter lifetime than other pump tubes. For pumping of organic solvents immiscible with water the displacement bottle arrangement is therefore preferred. Silicone pump tubes are extremely elastic and show, in most cases, an excellent performance in peristaltic pumps. Thick-walled silicone material seems to be particularly suitable.

In order to check whether a certain solution can be pumped by a given pump tube, small pieces of each pump tube can be left overnight in the solution. The pump tube pieces are then examined. The importance of lubricating the pump tubes and certain pump parts has already been stressed. The lifetime of a standard pump tube may, if it is correctly treated, amount to several months. This assumes that the pump tube is not left stretched overnight in the pump or left with the compression cam or band closed for long periods of time when the pump is off. The flow rate of a new pump tube changes during the first hours of use. This change may be as much as 10 - 20% for Acidflex pump tubes, but it is much lower for the other pump tube types. Normally, a slight, continuous decrease in flow rate is observed as the pump tube grows older.

3.6   INJECTORS

A necessary requirement when performing an analysis based on flow injection is, by definition, to insert a defined and reproducible volume of the sample into the carrier stream. To this end injectors are most commonly used. But insertion of a sample can also be accomplished through interaction of two or more pumps (hydrodynamic injection). This section will describe the injection device as a component; injection techniques are dealt with later in this book. The functional principles of a typical injector will first be described. Fig. 3.8 shows a six-port injector.
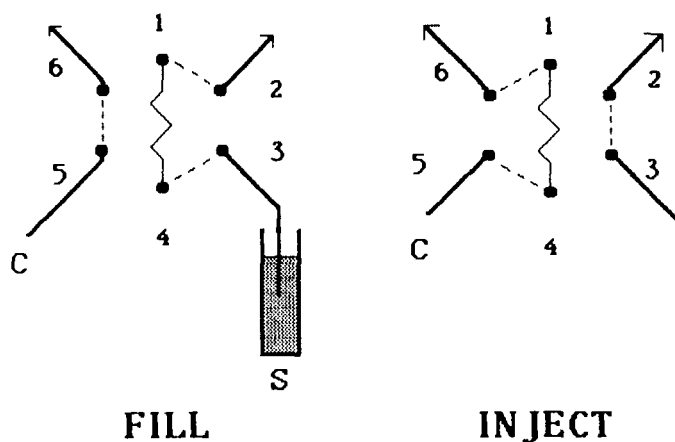
Fig. 3.8. Diagram of a six-port injector.

The sample loop is connected between ports 1 and 4. In the fill mode sample is aspirated into the sample loop and the carrier is pumped via ports 5 and 6 to the manifold. In the injection mode the port connections are rapidly switched so that the carrier stream sweeps through the sample loop and brings the sample as a discrete plug into the manifold. Note that sample aspiration continues until the sample flask is removed and replaced, preferably by a new sample or by a rinsing solution. The conduit volumes between two adjacent ports are small, typically a few microliters.

Several injector types have been developed and used for FIA and chromatography. In fact, most liquid chromatography valves are suitable for FIA (but not vice versa). The following aspects should be considered when choosing an injector for FIA:

1.   Volumes of liquid

The **injection volume** must be changeable at least in the range 30 - 200 μl. Once chosen, the volume should be perfectly constant.

The **sample volume** needed to fill the injector is inevitably larger than the injection volume but it should not be significantly larger. Almost all FIA papers present information about the injection volume but almost no FIA paper presents information about the total volume of sample needed to perform an analysis. This aspect is extremely vital, particularly when the sample amount is limited.

2.   Filling of sample

The most convenient way to fill the sample into the injector is by aspiration since a minimum of manual intervention is required. Filling from a syringe can be acceptable if very few samples are to be run, but it is totally unacceptable for large numbers of samples.

3.   Mode of activation

Automatic activation predominates and is definitely preferable. Manual activation often involves turning a movable part of the valve which is a difficult operation to carry out in a reproducible way. The activation period should be short. The carrier stream into which the sample is to be inserted is, for many injectors, interrupted during the activation period. This means that the proportions between the carrier and the reagent are changed temporarily. In some injector designs this effect has been decreased through implementation of a by-pass tube so that the carrier can continue to flow during the activation period.

From a functional point of view, there are two types of injectors, rotary valve injectors and syringe injectors. The design of rotary valve injectors, commonly used in liquid chromatography, and the predominating injector for FIA, has already been shown (Fig. 3.8).

Several versions of rotary valve injectors exist. Fig. 3.9 shows a refined six-port rotary valve injector provided with a by-pass to prevent the temporary interruption of the flow which occurs for most chromatography injectors.

a   b
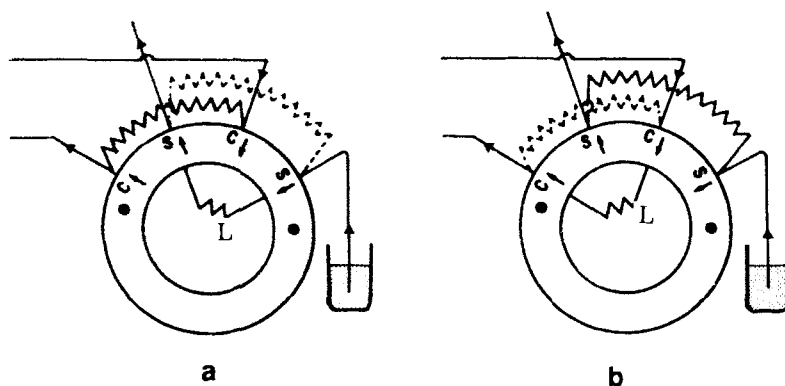
Fig. 3.9. Rotary valve injector with by-pass to prevent temporary interruptions of flow. a) fill position and b) inject position.

In the fill mode (3.9a), sample is aspirated into the sample loop and the carrier is led directly to the manifold. In the turn mode (3.9b) neither the carrier nor the sample stream is interrupted since the flows find their ways through the by-pass loops. It is important to fill the carrier by-pass loop with carrier solution before aspiration of sample starts, otherwise air will be introduced in the system.

In the injection mode (3.9c) the carrier transports the contents of the sample loop to the manifold. In all modes the by-pass loops are open but only in the turn mode is liquid flowing through them. The hydrodynamic resistance is essentially much larger in the by-pass loops (0.35 mm i.d.) in comparison with all other conduits connected to the injector (0.5 - 0.9 mm i.d.).

The principle of the second injector type, the syringe injector, is illustrated in Fig. 3.10.
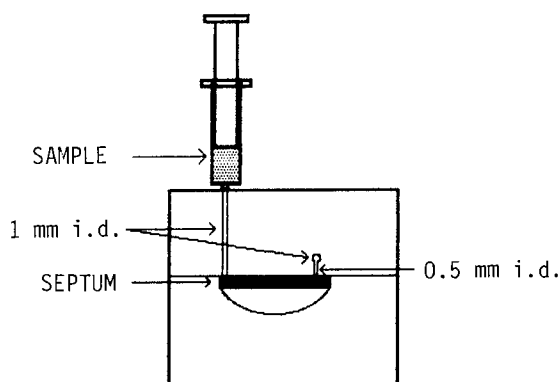
Fig. 3.10. Syringe injector.

An exact volume of sample is filled into the syringe and injected manually. A certain degree of technical skill by the operator is required. This type of injector was frequently employed in the pioneering phase of FIA but it now has considerably less popularity.

The exact volume of the injected sample can be determined by calibration. A dye, preferably bromothymol blue, is injected into the FIA system. In this case a borax solution is used both as carrier and as reagent to maintain a constant pH. No detector is needed at this stage. The outlet stream from the manifold is collected in a volumetric flask, see Fig. 3.11.
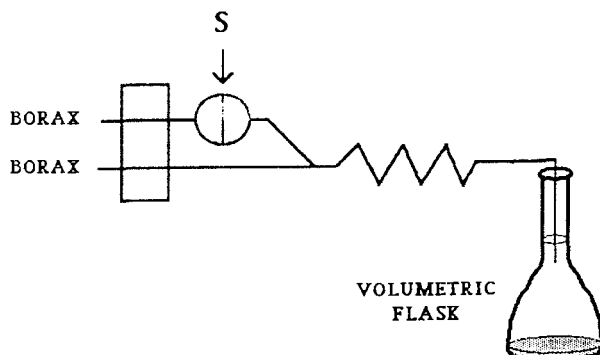
Fig. 3.11. Experimental set-up for evaluating injection volume.

When the entire sample zone has left the FIA system, and thus has been collected, the flask is made up to volume with borax solution. In similar volumetric flasks known volumes of the dye solution, for example 50, 100 and 200 μl, are portioned. All flasks are made up to volume with the same borax solution. The absorbances of calibration and sample solutions are measured, a calibration graph is constructed (it should be linear), and the sample volume is evaluated.

3.7   MANIFOLDS FOR GENERAL PURPOSE USE

The manifold is the focus of the physical mixing and the chemical reactions which take place in an FIA system. The manifold design can vary considerably depending on the conditions defined by the method. During the development stage of a method, it is desirable that manifold modifications can be made rapidly and easily to save time. On the other hand, once a manifold design has been developed and approved for routine purpose, a fixed, rigid structure is desirable.

In practice, the simplest possible manifold configuration is illustrated in Fig. 3.12.
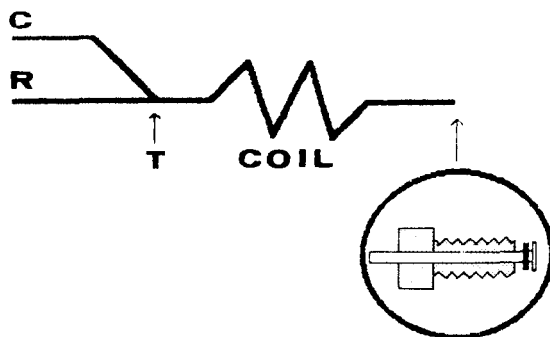
Fig. 3.12. Simple FIA manifold. C = carrier, R = reagent, T = mixing tee.

It consists of a tee for the merging of the carrier and reagent streams and a coiled reaction tube. At the tube end a connector of some kind is furnished to provide a convenient interface with the detector. Since the sample dispersion process predominantly occurs in the manifold, the design of the latter is delicate. As previously described, a suitable material for the sample and reagent connection tubes leading up to the tee is PTFE. The inner diameter of these tubes is usually in the range 0.5 - 0.8 mm. Fig. 3.13 illustrates some typical tubing connector (tees) configurations.
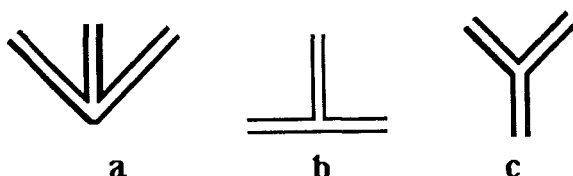
Fig. 3.13. Typical tubing connection (tees) used in FIA.
a) W, b) T, and c) Y configuration.

In most cases, the geometry of the tee (T or Y) is, in most cases, not critical as long as the bores are smooth and of constant inner diameter, preferably 0.5 - 0.8 mm i.d. As a general rule, cavities and/or constrictions should be avoided, particularly at the merging point. The reason for this is that irreproducible turbulence might occur. From a reliability point of view, the tee configuration in Fig. 3.13a is favored.

The reaction tube is situated between the tee and the detector, see Fig. 3.12. It is, in most cases, made of PTFE. The ends of the tube are flanged and provided with standard connectors and washers of viton rubber, see Fig. 3.14.

The tube mouth (or trumpet) must be symmetrical and the tube wall thickness must be even. Some skill has to be developed before satisfactory results are obtained. Ready-made tubes with washers and connectors can be purchased from most FIA manufacturers.
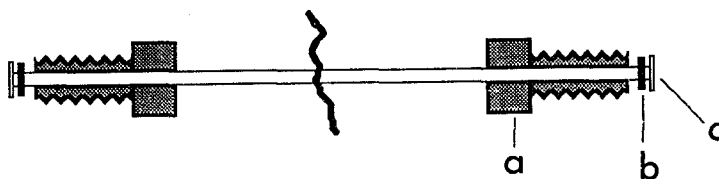
Fig. 3.14. Standard tube end configuration.
a) standard connector, b) washer, and c) flanged tube end.

The length of the reaction tube is normally in the range of 10 - 100 cm. A suitable coil diameter is 25 - 40 mm. The tube is coiled not only to save space and keep the system neat but, more importantly, to induce a secondary flow. The establishment of the secondary flow due to centrifugal forces restricts the amount of axial dispersion which will occur. The exact mechanism and optimized conditions for the establishment of the secondary flow are not clear. However, coiling the tubing does minimize dispersion.

Fig. 3.15 shows a commercial standard manifold (Chemifold II, Tecator, Sweden).

CHEMIFOLD™ TYPE II

U S Patent 4.224.033 U S and foreign patents pending
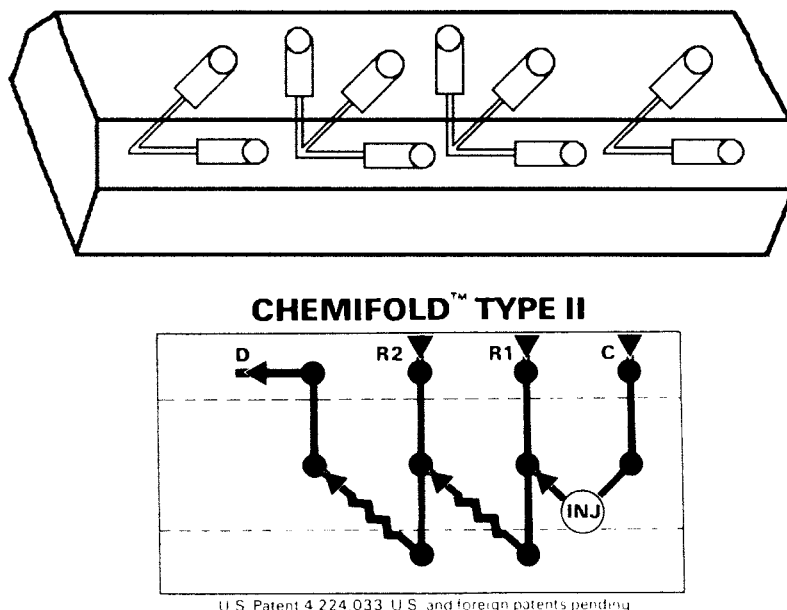
Fig. 3.15. Commercial FIA manifold for a method that requires two reagents.

The manifold is made of plexiglass. Standard coil lengths are 30 and 60 cm and the inner diameter is either 0.5 mm or 0.7 mm. Two coils can be connected using a standard low pressure union. A piece of sandpaper is included to improve the grip on the PTFE tube when a pump tube is to be connected. Also, a piece of silicone tube is provided to be used as a sleeve when a small bore pump tube and a PTFE tube are to be connected. This type of manifold is also available in a solvent resistant material for applications requiring non-aqueous solutions.

In 1984 Ruzicka and Hansen (3) introduced "integrated microconduits" for FIA in an effort to accommodate injector, manifold, and detector in one rigid unit. The mixing channels were engraved in small, transparent PVC blocks (70 x 45 x 10 mm) and closed by a plate or a plastic film. A basic microconduit unit is shown in Fig. 3.16.
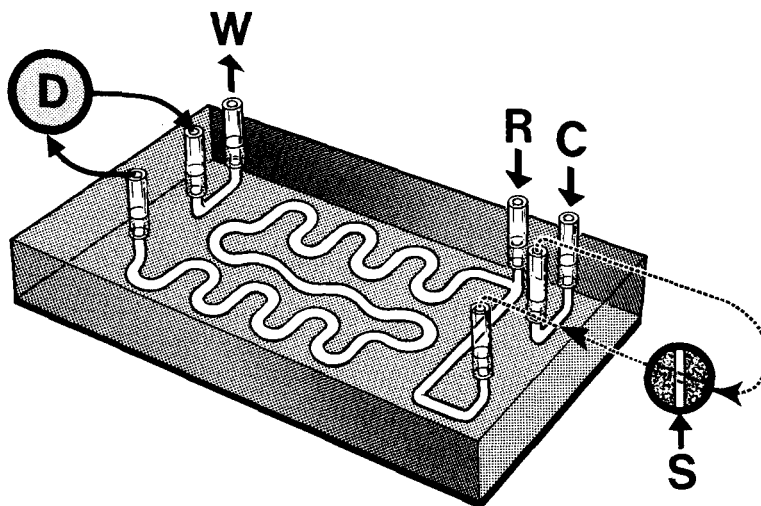
Fig. 3.16. Basic microconduit for FIA. R = reagent input, C = carrier input, S = external injector, D = detector connections, W = waste output.

This particular microconduit does not include an injector and detector. The length of the channel from the confluence point with R to the first detector point is 15.6 cm. This should be compared with the tube lengths of 30 - 60 cm which are typical for the flexible manifolds. Almost identical sample dispersion characteristics were observed for a 75 cm coiled tube, i.d. 0.5 mm, and the 15.6 cm engraved channel of the unit in Fig. 3.16 (3).

The cross-sectional area of the engraved channel is semicircular and about 0.8 mm^2. Information about flow dynamics in such channels is virtually nonexistent but possibly the radial mixing properties are improved over the corresponding properties of circular channels.

There are many advantages with integrated microconduits. They are rigid and therefore suitable for routine applications. The channels are protected from external influence that are mechanical in nature in contrast to a coiled tube for which the flow characteristics may be disturbed or altered when it is touched. They are small, compact, and easy to connect in an FIA system. However, a large number of different microconduits is required during method development since the entire unit has to be exchanged when, for instance, a systematic variation of the mixing coil length is performed. Strongly alkaline solutions and organic solutions might present problems with the seal between the closing plate or film and the engraved plate, but these problems can probably be mastered if an alternative sealing arrangement is worked out.

3.8 MANIFOLDS FOR MIXING

For most applications the mixing of sample and reagent in the manifold tube or channel occurs reproducibly and without complications or surprises. In other words, coiled tubes with an inner diameter of 0.5 - 0.7 mm and a length of 30 - 100 cm will do in most cases. Yet, the mixing process is of fundamental importance for the FIA user since there may be situations when it is desirable to improve and enhance the mixing efficiency. Two situations will be given as examples.

In the first example, the samples are assumed to have a high salt content. An immediate corrective measure for this problem is to use a carrier stream having about the same salt concentration as the samples. The reagent solution, which is normally rather dilute, can eventually be provided with an inert salt to match the salt content of the carrier and sample. In this way variations in the salt concentration are decreased or cancelled out. Still, the analyst might have some concern about a satisfactory mixing of the solutions in the coil since salt solutions are known to channel and to form unstirred layers.
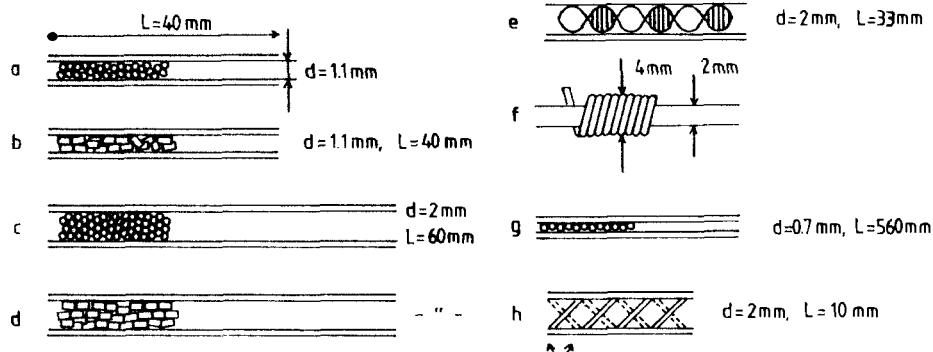
Fig. 3.17. Manifold designs for the optimized mixing of ethanolic reagents and aqueous samples (4). Mixers: a) Solvaflex tubing filled with glass beads (0.5 mm); b) Solvaflex tubing filled with small pieces of PTFE tubing (0.5 mm); c) PTFE tubing filled with glass beads (0.5 mm); d) PTFE tubing filled with small pieces of PTFE tubing (0.5 mm); e) PTFE tubing with a metal helix inserted; f) tightly coiled PTFE tubing (0.5 mm i.d.); g) PTFE tubing filled with a single string of glass beads (0.5 mm); h) magnetic stirrer, PTFE tubing with 6 pieces of iron wire.

In the second example, the samples are assumed to contain ethanol. Also in this case carrier and reagent solutions can be modified to conform with the sample matrix. For both types of samples, undesired phenomena may appear when a spectrophotometric detector is used. In the head and the tail zones of the sample plug, the variations in refractive index will cause focusing or diverging of the detector light beam. As a consequence, artifact peaks will be produced by the detector. These phenomena are called "matrix effects" among today's practicing FIA analysts. Such effects can be reduced by carrier and reagent matrix modifications, as already described, but to some extent also by improving the mixing efficiency of the FIA system.

Silfwerbrand-Lindh et al. (4) have investigated different manifold designs in an effort to optimize the mixing of ethanolic reagents and aqueous samples, see Fig. 3.17.

A combination of a Solvaflex tube (a type of pump tube) filled with PTFE pieces (Fig. 3.17b) and a tightly coiled PTFE tube, 2 m, i.d. 0.5 mm (Fig. 3.17f), was found to yield a satisfactory mixing in this case, see Table 3.2.

TABLE 3.2
The effect of different mixers on the system (4).

Number	Volume μl	Baseline noise, mm	Dispersion coefficient	Description
0	—	70	3.0	The system without mixer
1	30	15	3.3	Fig. 3.17a
2	60	6	3.3	Fig. 3.17b
3	40	13	3.2	Fig. 3.17c
4	60	12	3.5	Fig. 3.17d
5	60	30	3.5	Fig. 3.17e
6	140	10	3.4	Fig. 3.17g
7	20	35	—	Fig. 3.17h
8	200	5	4.1	Fig. 3.17f length 1 m
9	400	2	5.1	Fig. 3.17f length 2 m
10	400	2	5.1	Fig. 3.17f 2 coils, 1 m each, wound in opposite directions
11	200	—	4.1	Coil not tightly wound, 50 mm diam., 1 m length
12	400	12	6.5	Coil not tightly wound, 50 mm diam., 2 m length

The single bead stream reactor (s.b.s.r.) was described as early as 1981 by Reijn et al. (5). Dry glass beads with a mean diameter of 0.4 mm were carefully packed in a polyethylene tube, i.d. 0.6 mm, using a pipette and a funnel. The single bead string reactors showed less dispersion and enhanced micro-mixing (5). Several other studies have confirmed these findings.

An alternative to the filling procedure outlined above is to put the dry glass beads in a plastic bag. One of the ends of the tube to be filled, preferably a PTFE tube, is provided with a "stopper" of some water permeable foam plastic (certain types of packing material can be used). The other open end is then allowed to "dig" into the plastic bag so that the glass beads are packed into the tube in a zigzag pattern (tube i.d. 0.6 - 0.7 mm, glass bead i.d. 0.4 mm). When the entire tube has been filled the open end is sealed with a similar foam stopper. Thick-walled silicone tube pieces can then be used as sleeve connectors at each end of the filled tube. Fig. 3.18 shows the expected result of this procedure.

ENLARGED
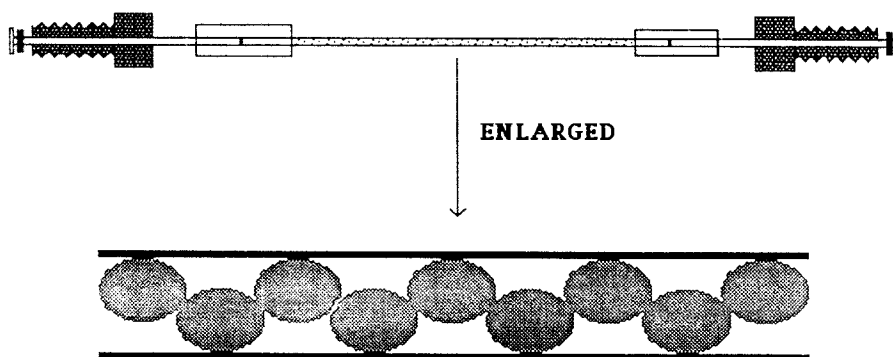
Fig. 3.18. Pearl string reactor.

Knitted or 3-D coils have been suggested and tested for postcolumn chromatography (6) and they have also been applied in FIA (3). Microline tubes are preferably selected for this purpose since this tube type permits knitting. A knitted coil is shown in Fig. 3.19.
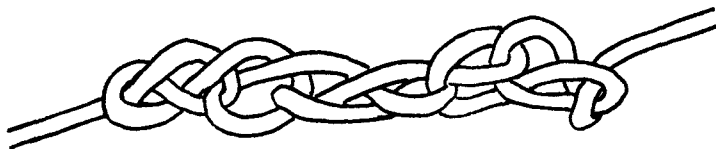
Fig. 3.19. Knitted coil reactor.

The sample dispersion in the axial direction is much less in a knitted coil compared to that in a straight tube of the same length and inner diameter. Whether the mixing ability is simultaneously improved to a corresponding degree can only be empirically studied for each individual application as was done for a number of other manifold configurations (4).

3.9 MANIFOLDS FOR DILUTION

In Chapter 2.7 factors effecting the sample dispersion (i.e. dilution) were discussed. The controllable dispersion was recognized to be a cornerstone for the FIA technique. The influence of coil length and diameter, sample volume and differential flow rate was described quantitatively and will not be repeated here especially since the conclusion was that the degree of sample dilution could not be varied by more than a factor of 10 using these variables. From a hardware point of view the components utilized to achieve dilution are the common standard components: mixing coils, injectors with variable volumes and pump tubes. These components have already been described. A specific component that deserves some attention here is the mixing chamber. An active mixing chamber consists of a uniform inlet tube, a cylindrical cavity accommodating a magnetic stirring bar, and a uniform outlet tube. A passive mixing chamber is designed similarly but lacks in-line moving parts. However, it may contain a static mixer such as a PTFE spiral or flake placed in the extended flow path. The volume of the cavity influences the degree of dilution. Since the sample is extensively dispersed in the axial direction the sampling frequency will be reduced correspondingly. It is therefore advisable to combine the implementation of a mixing chamber with the zone sampling technique using a two-channel injector.

Sample splitting and dialysis can also be utilized in order to dilute a sample. Table 3.3 gives a rough overview of the different dilution techniques.

TABLE 3.3

Dilution degrees (expressed in terms of dispersion coefficient) achieved by different techniques and manifold configuration where - means probable and . means possible.

Manifold/ technique	Dispersion coefficient, D				
	1	10	100	1 000	10 000
Variation of					
- sample volume	. . . . - - - -				
- coil length	. . . . . . -				
- coil diameter	. . . . -				
- C/R ratio	. . - - - - - - - . .				
Mixing chamber	. . . . . -				
Zone sampling	. . . . - - - - - - -				
Sample splitting	. . . . . - - -				
Zone sampling and sample splitting			. . . . . - - - - - - - - -		
Dialysis		. . - . .				

The normal working range for each manifold/technique is denoted by the total set of . and - signs. Within each range variation of D may often be accomplished in a convenient way. For example, variation of sample volume is in most cases made by an exchange of loops with different volumes. A change of the sample volume from 200 μl to 40 μl gives rise to about a threefold increase of D. This range is denoted by — in Table 3.3. The relative position of the — range within the total range depends on the choice of other manifold parameters such as coil geometry, flow ratio and flow configuration. All these parameters are interrelated with respect to their ability to influence the D value. With the zone sampling technique variation of D is easily accomplished by letting different times elapse between the two injections. Thus, a large variation of D can be performed without making any hardware modifications. The situation is quite the opposite for the mixing chamber technique. For a given mixing chamber design, a change of D can only be performed through variation of other parameters such as sample volume and carrier/reagent flow ratio. However, with a set of different mixing chambers having different volumes, manipulation of the D value is possible using this parameter only.

3.10   MANIFOLDS FOR GAS DIFFUSION

A gas diffusion cell is used in an FIA system to transfer a gaseous compound from one stream to another stream. The two streams are separated by a gas permeable membrane. Fig. 3.20 shows a common gas diffusion cell design.
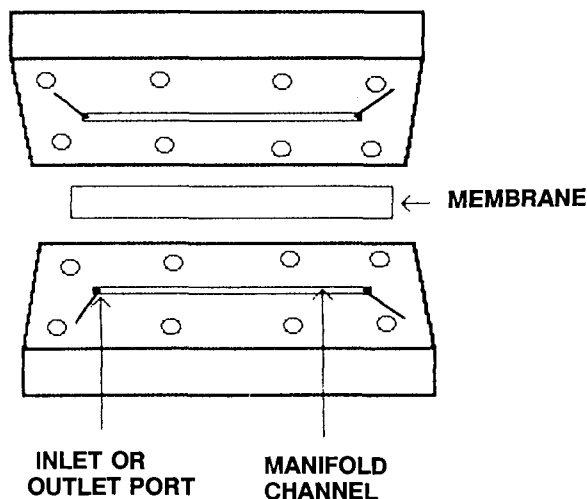
Fig. 3.20. Depiction of a gas diffusion cell.

Usually, the gaseous compound is either formed in the FIA system due to an induced chemical reaction or is present as the analyte of interest. The membrane is generally made of PTFE. Originally, plumber's tape was used but today porous PTFE membranes with a defined and uniform porosity are utilized. Very few fundamental studies of cell designs and membrane properties have been published. Cells with circular channels separated by circular membranes have been adapted by some FIA manufacturers (FIAtron, Tecator's Aquatec).

The lifetime of the membrane is largely dependent on the nature of the samples. Rain water samples do not affect the membrane at all and may permit daily analysis during several months without changing the membrane, while for soil samples it may be necessary to change the membrane every day. In addition, sharp edges on the engraved channels may damage the membrane during the mounting procedure. The membrane thickness, is in most cases, less than 0.5 mm. The membrane must not be excessively stretched when it is mounted since such handling will alter the diffusion properties and shorten the lifetime.

3.11 MANIFOLDS FOR DIALYSIS

Dialysis is an important sample pretreatment step whereby particles and large molecules in the sample can be removed. The sample is injected into a carrier stream which passes along a dialysis membrane. At the other side of the membrane a recipient stream (distilled water or a reagent) takes up the constituents passing through the membrane. The membrane is placed in a cell working on the same principle as the gas diffusion cell. In fact, many gas diffusion cells can be used for dialysis if a dialysis membrane is used instead of the porous PTFE tape. However, dialysis membranes do not possess the same sealing properties as the PTFE tape so leakages may appear. Rubber plates with engraved channels have therefore been used in the design of a dialysis unit, Fig. 3.21.

Groove pattern
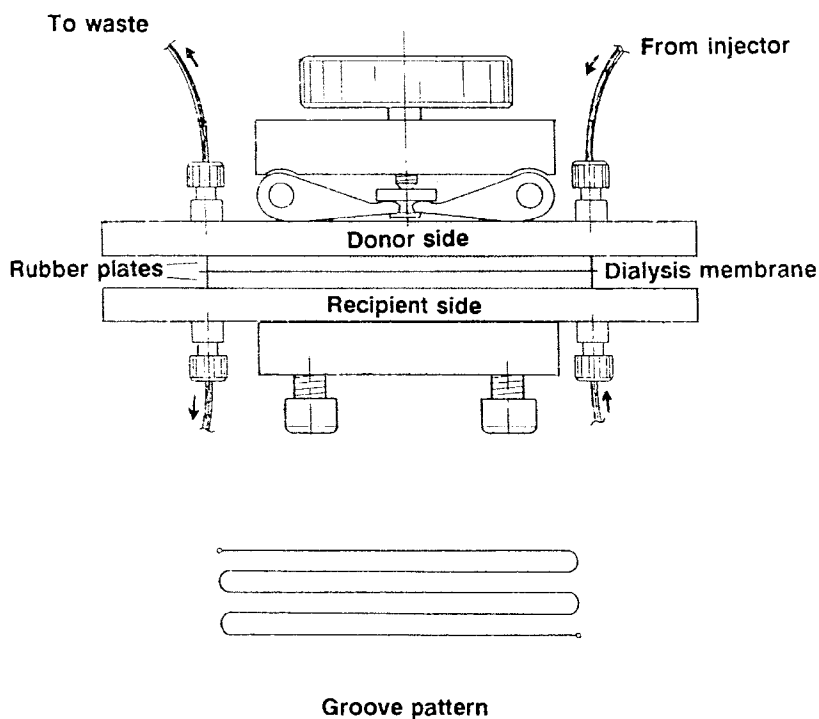
Fig. 3.21. Depiction of a dialysis manifold.

Only a fraction of the analyte penetrates the dialysis membrane and enters the recipient stream. The excess is expelled to waste. A substantial dilution of the originally injected analyte occurs. If the sample contains large molecules, colloid particles, and solid matter, the dialysis membrane acts as an efficient filter. The dialysis membranes must be soaked before they are installed. Most manufacturers supply presoaked membranes packed in closed plastic envelopes. The membranes are provided with a stiff frame so that the mounting can be performed safely. The molecular cutoff is usually 10,000 Daltons for standard dialysis membranes.

3.12 MANIFOLDS FOR LIQUID-LIQUID EXTRACTION

In a liquid-liquid extraction process, the sample constituents to be detected are transferred from their original matrix to an entirely new and different matrix. The merits, theory and applications of such a matrix modification are discussed in Chapter 6.1.

When the classical liquid-liquid extraction principle first was applied and adapted to FIA a rather complex design resulted (7), Fig. 3.22.
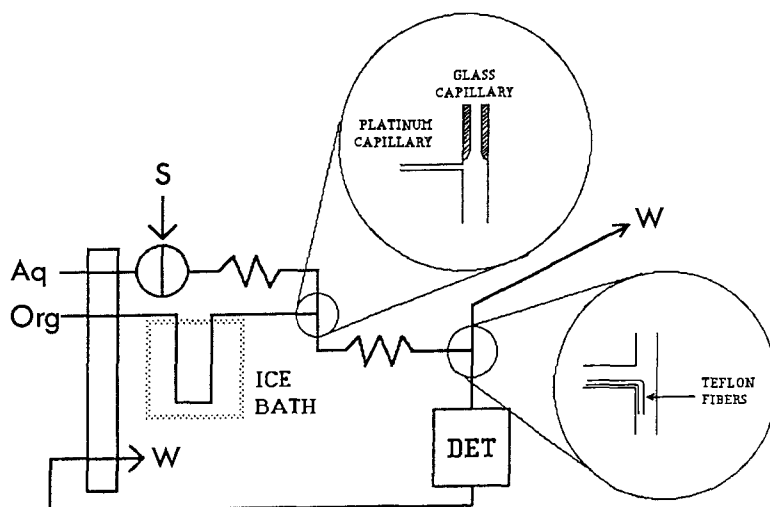
Fig. 3.22. Early extraction manifold design. Aq = aqueous phase, Org = organic phase, S = sample, DET = detector, W = waste. Only a fraction of the organic phase is passing through the flow cell.

The sample was injected in an aqueous carrier which was segmented with chloroform. The extraction took place in a PTFE coil. After phase separation a fraction of the organic phase was led through the flow cell in the spectrophotometer.

Four main hardware problems were recognized, namely the delivery of organic solvent to the system, solvent segmentation, selection of extraction coil material and dimensions, and, finally, separation of the two immiscible phases.

The pumping of organic solvent required special pump tubes, Acidflex tubes. Since no lubrication was performed (according to the recommendation in 3.5), friction heat developed and vapor segments appeared in the organic stream. To prevent formation of solvent vapor the organic stream was chilled in an ice bath. Soon it was discovered that the ice bath could be omitted completely if the pressure inside the system was increased. This was done through the implementation of a restriction coil at the waste outlet. Today, the displacement bottle technique, described in detail in 3.5, is almost exclusively applied for delivery of organic solvents immiscible with water.

The original segmentor (7) was made of a glass/steel fitting (Technicon A4) comprising one glass capillary inlet for the aqueous phase and one glass tube outlet for the segmented stream. The outlet tube was furnished with two inserted PTFE tubes so that its volume and its glass surface area could be varied just by displacing the PTFE tubes longitudinally. In this way the phase segment sizes could be changed conveniently. The requirement of a good segmentor is that it should produce even segments of alternating organic and aqueous phases and that the size of the segments can be varied in a controllable way. For some applications standard T-fittings of PTFE or polypropylene can be used as segmentors. An advantage with these molded fittings is that the bores are smooth. Machined fittings often contain small particles of material remaining which may give rise to a disorganized segmentation pattern. A disadvantage with these standard fittings is that the segment size cannot be varied.

Kawase (8) has studied different segmentor angles, i.e. different angles between the ingoing organic and aqueous streams and the outgoing segmented streams. No significant difference could be observed for the investigated configurations, namely T (90°), Y (30°) and W (45°). As a general rule the cavity size within the segmentor influences the segment size while the bore smoothness together with pump pulsation influence the segment regularity.

The most frequently used extraction coil material is PTFE with an inner diameter of 0.5 - 0.8 mm. It is of great importance that the phase which is carrying the analyte originally (in most cases water) does not form a film in the extraction coil. The reason for this rule of thumb is that the analyte constituent(s) in the injected sample then will be subjected to undesirable dispersion, a phenomenon which is discussed in detail in Chapter 6.1.

The component used to separate the two phases constituting the segmented stream is a critical component. Consequently, a variety of separators has been developed and described in the literature. Phase separators can be divided into three main groups: density separators, two-material separators and membrane separators.

The density separator consists of a chamber in which the two phases are allowed to settle and separate. The desired phase is removed either at the top or at the bottom.

The two-material separator contains, as the name indicates, two different materials which have different affinity to the two phases. Glass-PTFE is a common combination. A strip of PTFE is inserted in a glass T-fitting so that this material is present in two of three outlets/inlets. The T is then turned so that the segments of the phase possessing the strongest affinity to the PTFE material are guided downwards when this phase is the heaviest one of the two, and upwards in the reverse density case.

The most versatile separator type is the membrane separator which frequently utilizes a PTFE porous membrane, see Fig. 3.23.
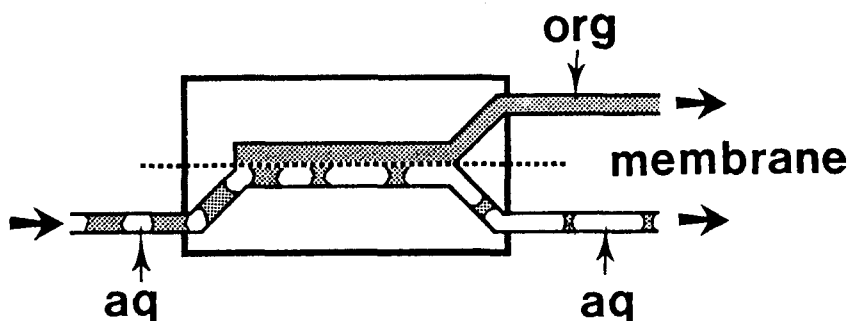
Fig. 3.23. Membrane separator for extraction.

The Figure shows the principle of a grooved membrane separator as it was designed originally (9). The dimensions of the grooves were 35 x 2 x 0.3 mm (length, width, depth). Cylindrical membrane separators have also been suggested (10).

The most important properties that should be considered for a separator used in an FIA system are its separation efficiency and its influence on sample dispersion. The separation efficiency should be close to 100% but it might be equally important that 0% of the undesired phase is allowed to be present in the collected fraction. If a spectrophotometer is used as a detector small water droplets in the collected organic phase may accumulate in the glass flow cell giving rise to drift and irreproducible results. Sample dispersion inevitably results if the "dead volume" of the separator is too large.

The complex flow pattern of FIA based extraction systems has definitely frightened many potential users of this technique for routine purposes. Consequently, a more user-friendly module has been designed (11). Its principle is shown is Fig. 3.24.

C: carrier
R: reagent
O: organic phase
W: waste
Inj: injector
D: detector

1: mixing conduit, aqueous phase
2: segmentor
3: extraction coil
4: separator, "donor' half
5: membrane
6: grid
7: separator; "recipient" half
8: separator steel cap
9: rinsing stopper
10: shut-off valve, rinse system
11: PVDF plate
12: rubber gasket
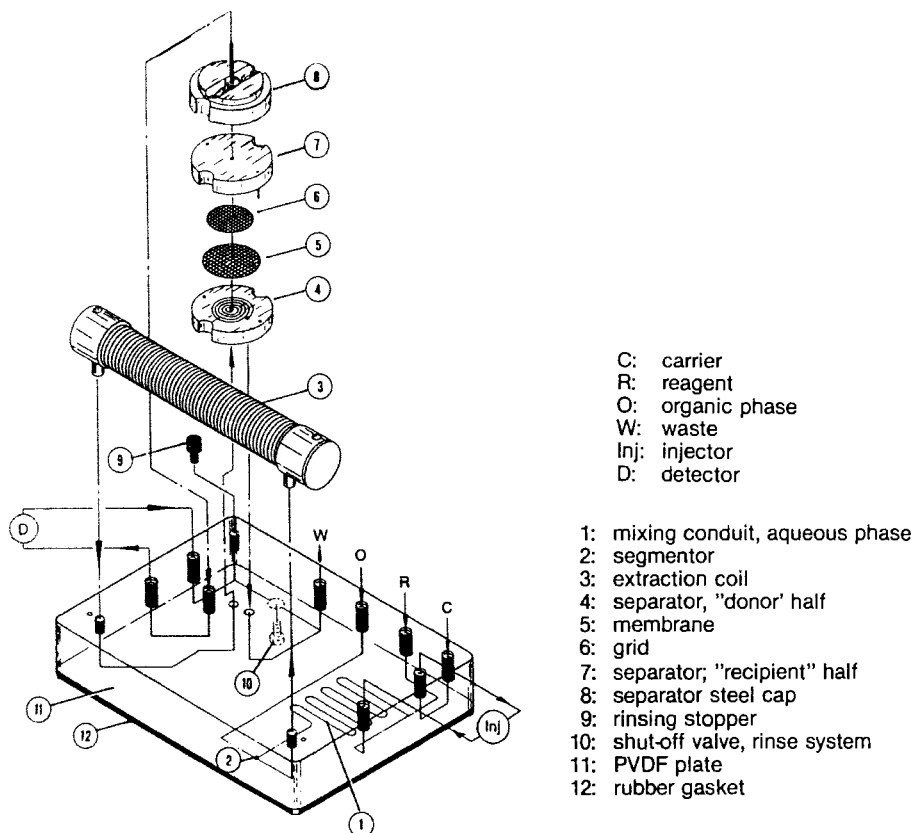
Fig. 3.24. Exploded view of an extraction module based on integrated conduits.

The mixing tee for C and R, the mixing coil and the segmentor are engraved in a PVDF body, 12 x 9 x 2 cm^3. The engraved channels are sealed with a sheet of inert rubber. The extraction coil (2 m; 0.5 mm i.d.) is detachable. The segmented stream enters the lower separator half (4 in Fig. 3.24) via a vertically drilled hole and hits the circular membrane at a 90° angle. A large fraction of the organic phase penetrates the membrane immediately while the aqueous phase is repelled. The remaining fraction of the organic phase penetrates the membrane as it flows through the circular groove providing for an efficient phase separation. A porous PTFE membrane with a porosity of 1 μ, is supported by a PTFE-coated steel grid. The acceptor chamber for the organic phase is underneath the upper separator half (7 in Fig. 3.24.)

During start-up and shut down of the system water droplets may accidently enter the flow cell so that an aqueous film is formed giving rise to continuously increasing or unstable baseline values. A rinsing procedure can then be applied, see Fig. 3.25.
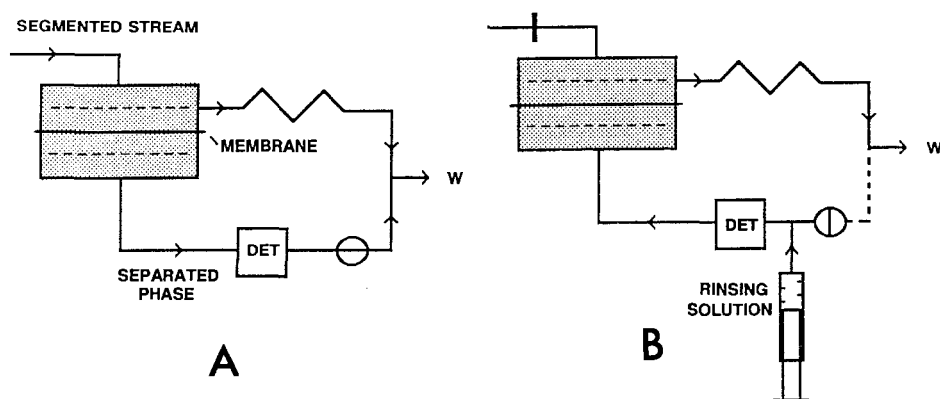
Fig. 3.25. Functioning of the rinsing system: (A) system in run mode; (B) system in rinse mode. The detector flow cell and the separator are rinsed with ethanol provided from a syringe. DET = detector, W = waste.

A commercial extraction module has been developed (Tecator 5106 Extraction Module) based on the published application and design data (11).

For coarse liquid-liquid extractions of concentrated samples a simplified extraction system can be used (12), see Fig. 3.26.
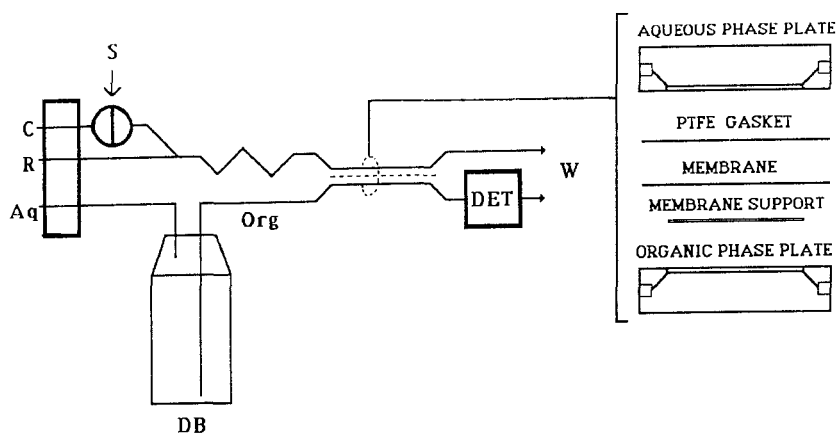
Fig. 3.26. Flow scheme of an unsegmented extraction system. Streams: C = carrier, R = reagent, Aq = water fed to displacement bottle (DB), Org = organic phase. S = sample, W = waste, DET = detector.

No segmentation takes place. The aqueous and the organic streams contact each other in the extraction cell. The dimensions of the organic groove is 40 x 2 x 0.8 mm (length, width, depth). This groove is filled with porous polyethylene to prevent collapsing of the membrane. The PTFE gasket on the aqueous side serves the same purpose. The design of this unsegmented extraction system is analogous to gas diffusion and dialysis systems. It is noteworthy that the extraction efficiency is comparable to typical gas diffusion or dialysis efficiency values, 8 - 18 %.

3.13   COLUMN REACTORS

Many FIA applications are based on chemical interactions and reactions between the analyte in the liquid sample and a solid material. The analyte is converted to a species which can be detected more conveniently than the analyte itself. In other situations, interfering substances in the sample are converted or trapped on a stationary solid material in the flow path. The selectivity of the method is thereby enhanced. To accomplish this, reactors are provided with the solid material in question and implemented in the FIA system. In Chapter 6.4 the different selectivity enhancement and matrix modification techniques are described more thoroughly. In this section, only the hardware and the preparation of the materials are reviewed. The single bead stream reactor (s.b.s.r.) already described in Chapter 3.8 fulfills quite a different task, namely to promote physical mixing. Thus, the purpose with the s.b.s.r. is fundamentally different since no chemical reaction is involved. However, from a hardware point of view this type of reactor is very similar to the chemical reactors.

The hardware needed to accommodate the solid material to be used for the chemical reaction in the flowing system can be extremely simple. A piece of pump tube provided with glass wool stoppers is, in many situations, quite sufficient. Instead of glasswool, cigarette filter material or nylon screen can be used. More mechanically reliable designs involve tubes made of plexiglass or solid PVC. Typical dimensions are 1 - 10 cm in length and 1 - 4 mm in inner diameter depending on the capacity requirements set by the chemistry involved.

Some examples of column reactors and their intended use are given in Table 3.4.

TABLE 3.4

Some different types of column reactors.

Reactor type	Filling material	Intended use
Glass bead reactors	Glass beads	To promote physical mixing
Redox		
Cadmium reductors	Cadmium granules	Analyte reduction
Jones reductor	Amalgamated zinc	
Ion exchange		
Anion	Ion exchange resin	Analyte conversion
Cation	Chelating resins	Matrix conversion
	Alumina, Silica	Preconcentration, removal of interfering substances
Adsorption	C_{18} silica	Matrix conversion Preconcentration
Enzyme reactors	Immobilized enzymes on column material or on CPG (Controlled Pore Glass)	Analyte conversion Removal of interfering substances

The different types of column reactors applications are dealt with further in Chapter 6.4.

3.14 OTHER COMPONENTS

It is obvious that components other than those already described in this chapter can be used when designing an FIA system. In this section some selected components will be treated.

Automated sample introduction from a sampler is offered by almost all FIA manufacturers. The samplers are of the same type as those normally used for air-segmented systems and for flame atomic absorption spectrophotometers. The liquid

samples are transferred into individual vessels which are placed either in a circular turntable or in a square-shaped rack. The sampling time is predefined so that the injection loop becomes completely filled. The wash time, i.e. the time during which the sampling probe either resides in a wash bath or is aspirating air is also predetermined. The sum of the sampling time and the wash time constitutes the cycle time. The sampling frequency is easily obtained by dividing 3600 by the cycle time expressed in seconds. Advanced samplers can be programmed to pick up samples in any given order and as many times as desired (random access samplers). The most commonly used samplers for FIA work through the samples position by position on the turntable. Carryover is usually a minor problem for most sampler types. A wash solution can be aspirated in between the aspiration of different samples. Often aspiration of air during the wash cycle is quite sufficient. If sample volume is a concern when multiple analyses are performed, the pump aspirating the sample should be programmed to be stopped during the analysis time. Recorders and computers are usually connected to the detector and will consequently be described in Chapter 4. However, pumps and injection valve units which are very essential, stand-alone FIA modules are sometimes furnished with an RS 232 interface to allow remote control from a computer. This configuration might be beneficial for applications for which complicated start/stop and inject/fill time cycles have to be defined. In commercial FIA instrumentation pumps and injectors are usually operated via a microprocessor in the main unit to allow convenient programming of the different event steps in the analytical cycle. Of course, the main FIA unit may, in turn, be operated from an external computer. Recorders are exclusively connected to the detector.

In-line filters are useful components for sample treatment. Even if the aspiration tube of the injector is a narrow bore conduit (i.d. typically 0.5 mm) and consequently acts as a sort of filter, some sample types require implementation of an in-line filter in the sample inlet stream. The disadvantage with this arrangement is that underpressure may result when the aspiration flow rate is too high. This may give rise to air bubbles in the stream.

Temperature control is accomplished by accomodating the entire manifold including the detector in an air-thermostatted compartment or by immersion into a thermostatted water bath (the detector may not be submersible!). The objective in both cases is to maintain a constant temperature (often slightly above room temperature). But there are several applications for which it is necessary to provide a large amount of heat in order to speed up the chemical reactions. Also in this case, water thermostats can be used. A more elegant way is to use an aluminum block with grooves for the PTFE reaction coil. The aluminum block is then heated electrically and kept at a constant temperature. Commercial temperature control units for FIA are available from several FIA manufacturers. At high temperatures it is necessary to add a restriction coil after the flow cell to prevent formation of gas bubbles. Alternatively, a piece of porous PTFE tubing can

be inserted in the flow path. The life-time of this type of PTFE tube is, unfortunately, rather limited when real-world samples are injected into the FIA system. Modified debubblers of Technicon type can probably also be considered. The original Technicon debubblers cannot be used since dimensions and material (glass) are not suitable for FIA purposes.

3.15 COMMERCIALLY AVAILABLE FLOW INJECTION ANALYZERS

Tecator FIAstar 5020 System

This system is modular. The central unit is equipped with two independently programmable four-channel pumps. The manifolds (Chemifolds) are easily interchangeable as are the injection valves (one channel and two channel valves, variable volume). Programming of pumps and injector and setting of parameters are made by thumbwheels and via a keyboard. The microprocessor in the central unit allows automatic calculation, recalibration and digital presentation of results. Five evaluation modes exist: peak height, peak area, peak width, peak-to-peak (for stopped-flow applications) and signal evaluation at a preset time after injection. Stopped-flow and intermittent pumping applications can be adapted.

The 5032 Detector System has a separate optical unit which is placed inside the central unit and a detector controller unit. The optical unit is either a dual beam spectrophotometer (400 - 700 nm) or a single beam photometer (with filters in the range 360 - 880 nm). The detector controller has a built in 4-color printer/plotter which plots absorption spectra and analytical data. The 5017 Sampler (Hook & Tucker, Great Britain) has a circular tray accommodating 40 sample cups, 2.5, 4.0 or 8.5 ml. The sampler is activated from the central unit (and not vice versa).

The central unit accepts any detector signals in the range ± 1000 mV but requires that at least one peak has a deflection of minimum 15 mV to guarantee result evaluation.

Several manifold designs are available including gas diffusion, dialysis, thermostatting and solvent extraction. A filtering and degassing unit is included in the central unit.

Tecator FIAstar 5010 System

This system has a different central unit than the FIAstar 5020 System but uses the same modular units as described above. In a stand-alone operation the 5010 Analyzer (central unit), the injection and wash cycle times can be specified via touch buttons. Also the total number of samples in a batch can be set.

With a compatible PC the SuperFlow Software takes care of calibrations, concentration evaluation and result reporting. Real-time monitoring is also possible. Stopped-flow and intermittent pumping applications can be designed.

Tecator Aquatec System

This system is specifically designed for ammonia, nitrite, nitrate, phosphate and chloride analyses in water. It can be operated either fully automated or by manual injections from a "pipette". The basic configuration of the Aquatec System comprises an "analyzer" and a "controller". In the analyzer the samples are mixed with reagents in conduits engraved in rubber plates. The absorbance is measured in an integrated optical unit. Aquatec incorporates a microprocessor to take care of data management, evaluation and communications. The programs are self-instructive. A total of 15 calibration curves can be stored in the memory for different methods and ranges. Each curve can contain up to 10 standards. Each method has its own, dedicated cassette which contains manifold, pre-installed pump tubes and reagent bottles.

FIAtron laboratory equipment

A large variety of modules is available. The FIA-VALVE 2000 is an eight port rotary sample injection valve. The valve is designed for low pressure (up to 30 psi) applications; all wetted surfaces are made of PTFE. It has its own keyboard for programming of different functions. The FIA-VALVE 2500 is an eight port selector valve and designed similarly.

The FIA-ZYME Analyzer Modules combine immobilized enzyme technology with a linear electrochemical sensor. All analysis and control parameters are programmable from the front panel or remotely via the RS-232 serial I/O port. Methods for glucose, fructose, sucrose, lactose, starch, ethanol and L-lactate are available.

The FIA-TRODE Analyzer Modules have been designed for potentiometric detection. Modules are available for pH, gas sensing probes (NH_3, CO_2, NO_2, SO_2 from HNU, USA) and ion-selective electrodes (from Orion and HNU, USA).

The FIA-LITE Analyzer Module is a colorimetric detector and the FIA-DUCT Analyzer Module is a conductivity detector.

The SC-110 Sample Changer has a capacity of 114 samples (ISCO, USA). The samples are placed in racks.

The FIAtron Process Flow Injection Analyzer

This analyzer is customized for individual process control applications. It has gas-driven pumps and a sample handling system with a bypass filter. Standards and sample are distributed to the injection valve via selector valve. Detection principles are UV/visible spectrophotometry, potentiometry (ion selective electrodes) or fluorimetry. The sample flow rate is in the range 2 - 200 ml/min and the sample consumption 5 - 50 μl/analysis. Sampling frequency is typically 60 analyses/hour. The analyzer is relatively large, 1.8 m x 0.6 m x 0.4 m (HWD), in comparison with common laboratory flow injection analyzers.

The Lachat QuikChem System IV

This modular system consists of the following units: a rack sampler (ISCO, USA; previously a circular sampler, Hook & Tucker, Great Britain) a peristaltic pump (Ismatec, Switzerland), a central unit housing reaction modules, i.e. coils and mixing tees, holders for ion selective electrodes, optical units (UV and/or visible), two injection valves (Hamilton, Switzerland; previously Rheodyne, USA), a multichannel detector controller for the optical units, a recorder and a data station. Many of the modules are optional. The system can be composed to run up to four analytes simultaneously. Automated sample preparation steps such as dilution, concentration and clean-up can be performed. Typical start up and method switching times are maximum 5 and 15 minutes, respectively. The real-time report generates uncorrected raw data and concentration units. Formatted printout with user-specified enhancements can be obtained off-line.

The CEC MultiFlow Flow Injection Analyzer

This analyzer has an integrated sample tray with a capacity of 150 cups, 4.0 ml. The pumping system is based on gas displacement and the flow rate range is 0.5 - 5 ml/min. The reagent capacity is 300 - 900 ml. The analyzer can be connected to any flow through detector, for example electrochemical, UV/visible, ion selective electrode and fluorimetric detectors. Furthermore, it can be combined with atomic spectroscopy detectors such as ICP and AAS. The injection valve is based on the hydrodynamic injection principle. Sample treatment steps like dilution, reagent addition, extraction, heating, cooling, separation and ion exchange can be implemented. The 4200 Integrator (Spectra-Physics, USA) is used with a software package enabling simultaneous acquisition of two channels, automatic calibration, result evaluation and reporting and drift compensating.

Chemlab Flow Injection Analyzer

This instrument contains the following in one compact unit: a five-channel peristaltic pump, a PTFE injection valve, an enclosed chemistry compartment, a variable temperature heating bath, a simple dual fiber-optic colorimeter, a quartz-halogen light source and an output to recorder or microprocessor. The basic system comprises the analyzer and a recorder. Samples are injected manually and results are read from the recorder trace after interpolation from a calibration curve.

For automatic peak height analysis the analyzer may be linked to a BBC microprocessor. An 80-place automatic sampler can also be connected as an option. Two or more analyzers can be linked together to provide a multi-channel system.

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REFERENCES

1.   J. Ruzicka, E.H. Hansen and A. Ramsing, Anal. Chim. Acta, 134 (1982) 55. [189]

2.   K.K. Stewart, Anal. Chem., 49 (1977) 2125. [-]

3.   J. Ruzicka and E.H. Hansen, Anal. Chim. Acta, 161 (1984) 1. [456]

4.   L.C. Silfwerbrand, L. Nord, L.G. Danielsson and F. Ingman, Anal. Chim. Acta, 160 (1984) 11. [422]

5.   J.M. Reijn, W.E. Van der Linden and H. Poppe, Anal. Chim. Acta, 123 (1981) 229. [114]

6.   U. Neue, Chromatographia, 15 (1982) 403. [-]

7.   B. Karlberg and S. Thelander, Anal. Chim. Acta, 98 (1978) 1. [17]

8.   J. Kawase, Anal. Chem., 52 (1980) 2124. [107]

9.   B. Karlberg and L. Nord, Anal. Chim. Acta, 118 (1980) 285. [92]

10.   K. Backstrom, L.G. Danielsson and L. Nord, Anal. Chim. Acta, 169 (1985) 43. [608]

11.   Y. Sahlestrom and B. Karlberg, Anal. Chim. Acta, 185 (1986) 259. [933]

12.   Y. Sahlestrom and B. Karlberg, Anal. Chim. Acta, 179 (1986) 315. [742]

CHAPTER 4

DETECTORS IN FIA

4.1   INTRODUCTION

At first glance it would appear that the detector requirements commonly cited for HPLC are the same for FIA. However, this is not true for all detectors. It is the goal of this chapter to present the similarities and differences between HPLC and FIA detector requirements. To this end it should be realized that the purpose of HPLC is to separate and detect individual components of a sample, while FIA usually is used to perform chemistry on only one constituent and detect that analytical species. In both cases the function of the detector is the same, measurement of the signal produced by the analyte or analyte/reagent product. The parameters discussed include the physical detector configuration and the readout/software techniques currently employed. The question as to whether peak height or peak area is the best measurement of an FIA signal is also discussed. Finally, the more commonly used detectors for FIA will be described in detail.

4.2   DETECTOR REQUIREMENTS FOR FIA IN COMPARISON WITH HPLC

Present day HPLC detectors are on-stream, continuous flow monitors which measure some physical property, e.g., light absorption, voltage, and photon flux. In the past, HPLC systems employed low flow rates with sample sizes of 10 - 100 μl. Recent advances in HPLC have resulted in flow rates of 1 - 4 ml/min and small sample volumes of 1 - 5 μl. FIA usually utilizes similar total flow rates of 1 - 3 ml/min. Injected sample volumes are usually larger, 20 - 200 μl. However, some FIA detectors and methods can utilize larger samples and/or flow rates to produce increased signals. In an HPLC system each injected sample is significantly diluted during the process of separation of its different constituents. Consequently, HPLC detectors have been designed to be extremely sensitive. In an FIA system sample dilution is controllable and, therefore, the manifolds are designed to be the main contributors to the dilution of the sample. In conclusion, detector requirements for FIA are more relaxed in comparison to HPLC.

For HPLC it is very important to have well designed flow cells in which the separated bands remain resolved and no additional band broadening occurs. Cell volumes, formerly in the 18 - 75 μl range, are now more commonly 8 μl or smaller. The

term cell efficiency is used to define the ability of the detector cell to maintain the eluted sample components as sharp, separate bands with the cell contributing no additional dilution. This dilution would produce band broadening, peak height reduction, peak overlap, and/or peak tailing which all leads to a loss of resolution.

Band broadening will occur because, due to longitudinal diffusion, the same amount of solute will occupy a larger mobile phase volume, thus increasing the peak width. In HPLC band broadening must be minimized. Does it have to be minimized in FIA? In FIA band broadening first must be reproducible. The dilution caused by band broadening will only affect the detection limit of the FIA method. Therefore, minimizing band broadening is important if the detection limit is a critical problem.

The important question about band broadening deals with the detector output. Which measurement is affected by band broadening; peak height or peak area? The peak area is proportional to the total sample amount even when band broadening increases. Since the sample amount is unchanged, the area is unchanged. Peak height, on the other hand, decreases with increasing band broadening. In HPLC peak area is usually used to quantitate the sample components. This is due to the irreproducibilities of the column separation. In FIA the reproducibility is very good, therefore, peak height is usually used to quantitate the sample. The process of peak overlapping occurs in HPLC when the maximum of the peaks essentially remains at the same position, but their width increases so much that they actually enter into each other. Obviously, this is a critical problem for HPLC since the separated peaks would be remixed because of this overlap. Since no separations are carried out in a "normal" FIA system, peak overlapping is of no significance. When FIA systems are used to separate constituents, the objectives are quite different from those of HPLC. For instance, the analyte in FIA can be separated from the matrix by gas diffusion or liquid-liquid extraction. In neither situation does peak overlapping exist as a phenomenon or problem.

Peak tailing might occur if parts of the dectector cell are inefficiently flushed by the mobile phase in HPLC or carrier stream in FIA. Thus, solute molecules entering these areas will only be able to slowly leave them again. For both HPLC and FIA low detector cell dead volume is important. However, it is more critical for HPLC than FIA.

With this information in hand, does it appear that HPLC or FIA has the most demanding detector requirements? The physical requirements on HPLC detectors are more stringent than on FIA detectors. Therefore, in most cases, an HPLC detector can be used in FIA systems. Are there any differences between HPLC and FIA detectors that must be taken into account? The major difference between FIA and HPLC detectors is that FIA can use detectors that HPLC, from a practical viewpoint, cannot. But more importantly, FIA needs the detector and the readout device to respond and report the data at a much faster rate. Many HPLC detector systems come with software which does not allow for faster accumulation of data. The obvious result is that the FIA systems will have

peaks passing through the detector faster than it can respond. The result is nonreproducibility of the signal.

Essentially, any detector capable of flow through detection can be interfaced to an FIA system. Even instruments such as mass spectrometry, which are not usually considered "flow through", have been used as detectors in FIA. This single point makes FIA extremely versatile and valuable. However, the intrinsic power of FIA will only be realized when multiwavelength detectors are used in order to take advantage of the concentration gradient that is formed in the FIA sample plug.

Some special requirements for FIA detectors will be mentioned which relate to the stopping (and the restarting) of the flowing stream. If a stopped flow period is used, it is absolutely essential that no artifact transient signals appear since these can be interpreted as real peaks. Another important aspect to be considered when stopping the sample stream is that the section of the stream that is situated inside the flow through cell might be heated by the light source of the detector. If this occurs for a stopped-flow application, part of the sample zone will be leaving the detector during the stop period due to the expansion of the liquid. This effect can, to some extent, be prevented by feeding the outlet stream from the flow cell back into the pump. Thermostatting of the flow cell can also be applied. If optical fibers are used the light source can be located at some distance from the cell which means that the heating problem should not occur. If the stopped-flow period is used only to create a time delay, then the sample zone can be stopped before the cell.

Finally, something should be said about the use of recorders connected to the detector. During method development it is mandatory to use a recorder so that baseline drift, double peaks, peak distortion etc. can be observed. These pieces of information will give clues on how to optimize the system. Only when a method is completely established for routine determination can the recording be left out and replaced by computerized evaluation of peak height or peak area (integration). Integration is so commonly applied for HPLC that many detectors are provided with this option as a standard mode of evaluation. In the next section of this chapter some aspects of peak height versus peak area are discussed.

4.3   PEAK HEIGHT VERSUS PEAK AREA

Chromatography has traditionally used peak area as the means of quantitating detector response. It has been tempting for many researchers to carry on this tradition with FIA. In most FIA peaks, sample, reagent, and reaction products are all present, while HPLC peaks usually contain only the separated constituents. However, we should ask; why is peak area used for chromatography? Are the reasons given also good for FIA or

are there other considerations which take precedence? Peak area is used in chromatography because the physical and surface based properties of adsorption and/or size exclusion are not absolutely reproducible. The irreproducibility makes the use of peak area necessary. Peak area can be used since it assumes that the signal produced is proportional to the analyte concentration. This is a good assumption for the majority of HPLC systems. Peak height requires high reproducibility with respect to time; however, peak area does not. In FIA systems, the physical and surface based properties do not add irreproducibility in terms of time and peak broadening. Therefore, peak height becomes a highly reproducible measurement of analytical signal, typically ± 1%.

The FIA purists have an additional reason for using peak height. For the first time in analytical chemistry, a reproducible concentration gradient containing analyte, reagent and/or detected species can be obtained. The potential analytical application of the concentration gradient is very large. If peak area is used to quantitate the signal, all gradient information is lost. The gain in precision is minimal at best. Therefore, a wealth of information is neglected without any significant gain in performance. It should be pointed out that the gradient concept in FIA, which refers exclusively to the sample, is in no way related to the solvent gradient concept in HPLC. The latter refers to controlled changes in the bulk properties of the mobile phase, i.e. changes in solvent and solute composition.

Another aspect in the discussion of whether peak height or peak area is preferred in FIA relates to the kinetics of the analyte formation. The molecules in the head of the sample peak pass through the detector several seconds before the molecules in the tail of the sample. If the chemical reaction is slow and incomplete this time difference may be of importance. During these extra seconds the chemical reaction might approach completion or steady-state by some additional percent units. This means that fewer molecules (or ions) of R and S are needed in the tail section to produce a certain amount of RS in comparison with corresponding amount in the head section. The original S concentration and the concentration of the formed RS, are consequently not consistently related to each other across the resulting peak profile on the recorder paper. Usually this discrepancy is of no significance since calibration is made with standards covering the entire sample concentration range. Note that this kinetic aspect does not apply to normal HPLC since the different detected species are not chemically converted.

Some electrochemical detectors produce signals which are correlated to the logarithm of the analyte concentration. The relationship between peak area and analyte concentration then becomes very complex. Peak height evaluation is preferred in this situation.

Other detection modes are also used from time to time in an FIA system. In the case of FIA titrations, the elapsed time between two points with equal dispersion, one on the ascending side of the peak and the other on the descending side of the peak, is

measured. This time is correlated to the analyte concentration of the sample. In the case of stopped flow experiments, either the final increase in signal or the slope of the rising signal can be used to evaluate the analyte concentration.

The most interesting aspect of signal measurement in an FIA system is that any point on the FIA response curve can be used to evaluate the samples under investigation. Multiple calibration curves or time based measurements can thus be applied. At the current state of development, only the capabilities of the detectors and the imagination of the analyst have limited the FIA technique.

4.4   SPECTROPHOTOMETRY (UV AND VISIBLE)

For all practical purposes the commercially available UV-visible flow through detectors will easily accommodate FIA assuming that the response time of the detector/readout device is fast enough to measure and output the FIA signal. The real advantage of using FIA for spectrophotometric detection is the high degree of reproducibility. The fact that the signal from the sample does not have to be at steady state will increase sample throughput.

Most flow cells for optical spectroscopy are designed so that the source beam is passed through the longitudinal axis of the cell. This design maximizes the cell path. But does this design allow for the maximum amount of information to be collected? In this configuration the stratified sample zone is integrated over the entire optical path length and the detector yields a rapid response. In this viewing position the concentration gradient is present in the flow stream but all gradient based information is not recorded because of this integration. Basically, the longer and larger the flow cell, the less the amount of gradient information which can be obtained. If gradient observation with maximum resolution is the goal, then the source beam must pass through the entire width of the sample cell. Even then, an integration of the dispersed sample zone is observed. However, this integration is performed radially over a smaller area of the sample zone.

The actual flow cells are usually designed in a "Z" configuration (Fig. 4.1a). The transparent windows are made of glass (for visible only) or quartz (for UV and visible measurements). When using this cell the carrier stream should be fed from the bottom. This approach minimizes the amount of trapped air bubbles. A second alternative is to use the "U-shaped" cell design (Fig. 4.1b). Notice the "J" shaped entry tube into the cell compartment. It is critical that the cell has this configuration and that the solutions are introduced through this side in order to eliminate air bubble problems. A 10 mm cell path is standard and the cells are made of glass or quartz. These cells come with glass tubing extending from the top of the cell. The use of silicone tubing sleeves enables easy connection of the cell to the manifold tube and waste streams.
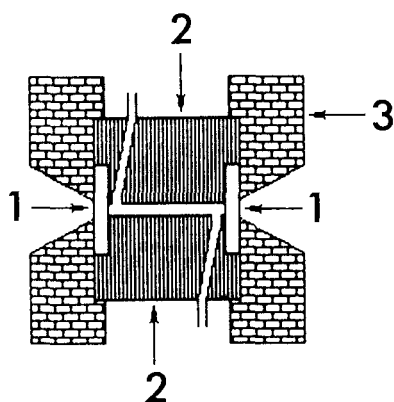
1 - transparent windows
2 - Teflon body
3 - cell housing
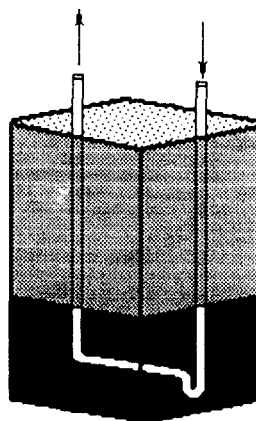
a   b

Fig. 4.1. a) The Z configuration flow cell b) the U configuration flow cell.

        In some designs the cell uses conventional connectors to attach the cell to the
manifold and waste container. In terms of simplicity, this latter approach appears to be
the best and least expensive. The diameter of the connecting tube and the flow channel
of the cell should be similar. However, not all spectrophotometers can be used with small
volume flow cells. With some designs the amount of incident light which will pass through
the cell is insufficient for the spectrophotometer to function correctly. The low intensity
of the incident light is usually caused by low power source lamps or optical bench
alignments which do not pass the source beam perfectly parallel to the cell cavity. The
result in the latter case is that the light is bounced into the side of the cell where either
additional stray light is created or the light is absorbed by the blackened walls of the cell.
        When microconduits are used the spectrophotometer light is guided to the cell
compartment using fiber optics (1). The returning light is collected and returned via fiber
optics. This approach can also incorporate a 10 mm cell path depending on the function
and sensitivity required.

The FIA system does not affect any of the basic traits of batch spectrophotometric methods. Beer's law will still be obeyed in the same range. The sensitivity of the method is about the same. Throughput and precision are increased. The actual detection limits will be comparable or better since the noise contribution due to human handling of the sample is eliminated. Without question, spectrophotometric detection is the most commonly used detection scheme for routine monitoring.

An exciting potential use of UV-visible spectrophotometry is the incorporation of diode array detectors into the FIA flow system. The high reproducibility of FIA will make the use of diode array detectors for multicomponent analysis, kinetics, and routine monitoring extremely easy to use. In particular, the use of multiple injection techniques like chasing zones (see Chapter 7) will be greatly enhanced by the power of the diode array detector. Background absorbance of the sample might be corrected as well as other effects normally created by the sample matrix. Multicomponent analysis would also be possible using multipurpose reagents. The practical potential of diode array detection remains to be exploited.

4.5 LUMINESCENT DETECTION

In this section the luminescent techniques; fluorescence, phosphorescence, chemiluminescence and bioluminescence are discussed. All four techniques are improved using FIA. The dispersion coefficient, D, for a manifold with these detector systems is usually in the medium category, D = 3 - 20. This is because the analyte must first be mixed with the reagent before the chemistry can occur and the signal observed. Most luminescent techniques/methods are not selective. Therefore, some type of selectivity enhancement technique is usually incorporated into these FIA manifolds.

FIA definitely improves the performance of chemiluminescence and bioluminescence detectors since it is ideally suited to maximize the analyte signal. Usually the light production is so rapid, In the milliseconds range, that quick mixing immediately followed by detection is critical to the sensitivity of the system. As a result, only reactions with long lifetimes can be used in batch methods. Adaptation of the mixing process to FIA allows for immediate detection at the point of mixing. Therefore, reagent/analyte complexes, which produce light on the milliseconds time scale or faster, can be used effectively.

A typical detection set-up is shown in Fig. 4.2. The luminescent reagent and the sample are mixed in front of a spiral T-cell which is positioned in front of a photomultiplier tube. In the case of the reaction of luminol with chlorine dioxide, light production is completely finished before the reagent/analyte complexes leave the spiral cell. The only negative aspect of this cell design is that the FIA peak is Integrated versus time. Therefore, no gradient information is recorded; however, the gradient is intact.
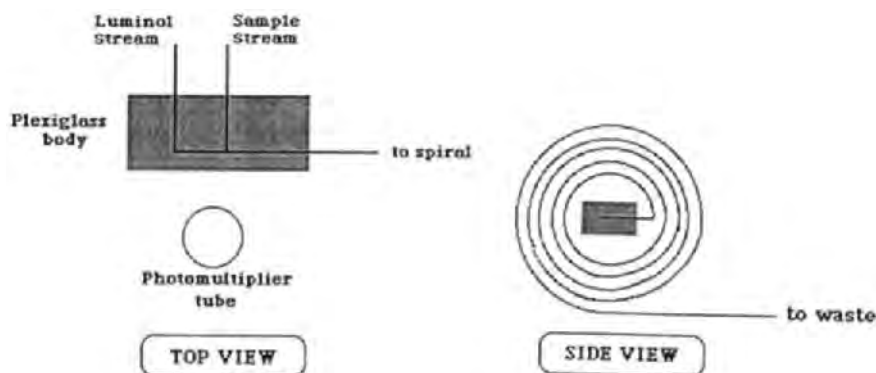
Fig. 4.2. A detector set-up for a fast chemiluminescent reaction product using FIA.

The major advantage of chemiluminescence systems is that they usually exhibit linear ranges that span several decades. It is typical for the linear ranges to be 1000 to 100,000 concentration units in width. This provides a great deal of flexibility to the analyst.

Using microconduit based designs, the luminescence is observed through a fiber optic which is placed at a point where the reagent and the analyte meet (1). In such a design there is less integration of the signal and, consequently, more gradient information is recorded.

For fluorescence based studies the commercially available HPLC detection systems are more than adequate. The classical design for fluorescence cells is to pass excitation light into one side of the flow cell compartment and observe the fluorescence at a 90° angle. Unfortunately, the effective cell path is small using this configuration. Other more efficient designs collect the majority of fluorescence emitted light using a concave mirror and focus it onto the detector.

The advantage of using FIA for fluorescence based measurements is that reproducibility can be significantly enhanced. Fluorescence is affected by environmental conditions more than colorimetry. The observed relative intensities can be dramatically affected by solution based properties like ionic strength, pH and interferents. FIA stabilizes the solution conditions thereby increasing precision. No quenching from oxygen will occur since oxygen can be eliminated from the closed system. For some applications a matrix modification step is performed in the FIA system to eliminate quenching problems. Using matrix modification it is possible to carry out the reaction chemistry under optimized conditions and to merge this pretreated zone with an optimized detection solution.

Phosphorescent based systems, though less frequently used, exhibit improved performance characteristics. The future for this technique in flow injection is limited, however, because many phosphorescent methods require low temperatures or viscous solutions.

4.6 ATOMIC SPECTROSCOPY

In principle, any atomic absorption spectrometer (AAS) or inductively coupled plasma (ICP) detector can be connected to an FIA system. In practice, AAS and ICP detectors are not easily moved around in the laboratory which means that the FIA system is brought to the AAS or ICP detector and not vice versa. The connection itself is relatively straightforward; the aspiration tube and the FIA manifold outlet tube are coupled together using a sleeve tube. For AAS, the aspiration tube can be removed completely from the nebulizer and replaced by the FIA outlet tube. The simplest possible FIA system that is connected to an AAS or ICP detector contains a pump, an injector and a single-line manifold. Compared with other detectors, relatively high flow rates are required for AAS, typically 4 - 8 ml/min. The flow rate of the liquid is usually adjusted to be equal or even slightly higher than the AAS aspiration flow rate. For ICP the flow rates are lower, 1 - 3 ml/min.

Sample volumes are in the range of 10 - 1000 μl. Such volumes give rise to reproducible transient detector signals, in other words, typical FIA peaks. When the sample is evaluated as peak height, a certain loss in signal is obtained in comparison with continuous aspiration. The aspiration usually continues until the steady state signal level has been attained, requiring sample volumes larger than 1000 μl. The signal loss is expected since the limited amount of sample used with FIA does not allow a steady state level to be obtained. Different flow and detector configurations will also behave differently with respect to the reduction in peak height. The flow part of the system must be carefully designed so that the sample plug in the carrier reaches the detector without being subjected to unnecessary dispersion. The volume and the geometrical properties of the aerosol chamber are other critical parameters in this context. Normally, the analyst has limited possibilities to influence the design of the aerosol chamber but one should be aware of the fact that there is quite a difference between different designs.

As previously mentioned, the continuous aspiration mode requires a minimum of 1000 μl to reach a steady state signal. Using FIA, about 60% of the steady state signal is obtained when only 100 μl of the sample is injected. If the sample supply is limited, the FIA approach for sample introduction is a highly recommended alternative to the conventional aspiration approach. The sample throughput depends on the sample volume injected; the smaller the sample volume the higher the sampling frequency and the larger

the loss in signal (evaluated as peak height).

The different merits of combining AAS and FIA will now be discussed in some detail. First, what can be gained by pumping the carrier continuously into the nebulizer of an AAS instrument? Actually, the FIA's continuous feed allows steady conditions to be attained inside the nebulizer. The feed rate is constant with FIA even if samples having different viscosity properties are injected. The changes in feed rate caused by the variations in sample viscosity is a recognized problem when sample introduction by aspiration is applied.

Is the selection of the FIA carrier stream crucial to atomic absorption detection? Normally, distilled water is used as carrier. But it has been shown that signal enhancement can be obtained for AAS detectors when an appropriate organic solvent is used as a carrier, for example methyl isobutyl ketone (MIBK), methanol, or acetone (2,3). An improvement factor of up to 8 can be accomplished for certain combinations of carrier/sample solvents in comparison with aqueous conditions (3).

What further benefits result from an interfacing of an FIA system? With FIA a variety of sample pretreatment steps can conveniently be performed, such as dilution, reagent addition, preconcentration, liquid-liquid extraction, analyte conversion and hydride generation. There are also situations when sample pretreatment becomes unnecessary with FIA. For example, high salt samples do not need dilution to prevent clogging of the nebulizer slot. Using FIA the burner slot is exposed less to the sample. The flame does not extinguish and the performance and operational efficiency of the analytical system is improved.

The manifolds needed to perform a variety of different sample pretreatment steps have been described in Chapter 3 except for the hydride generation manifold. Fig. 4.3 shows the original hydride generation system used by Astrom (4).
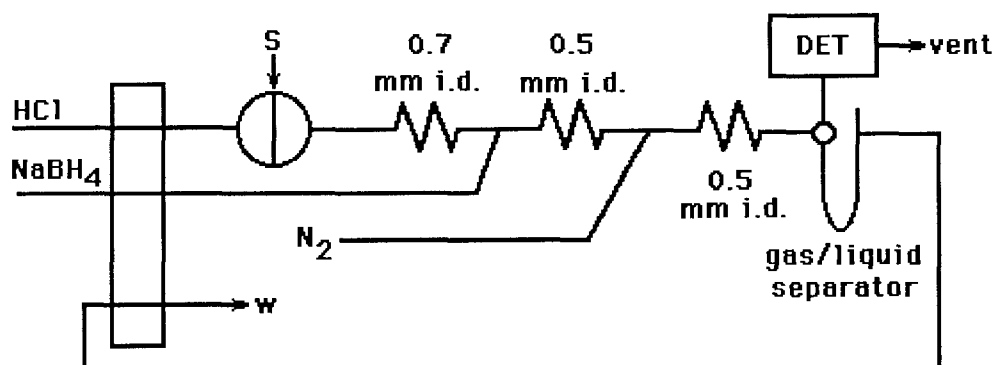
Fig. 4.3. Hydride generation manifold of Astrom. S = sample, DET = AAS detector, W = waste.

The U-type gas-liquid separator according to Vijan (5) was miniaturized to keep the volume as low as possible. The quartz cell was heated electrically to about 850 °C using Kanthal wires. Microporous PTFE tubes have also been utilized as gas-liquid separators (6) as well as dual phase gas diffusion cells of a more conventional design (7). In comparison with the original batch methods for hydride generation the interferences from transition metals decrease significantly when corresponding reactions are performed in an FIA system. This effect can be explained by the short residence time that the sample has with the interfering transition metal ions in the sample. The adsorption of a hydride such as arsine on the transition metal hydride is minimized. Another explanation might be that the short residence time for the hydride in the system prevents its decomposition which may occur in the acidic, liquid environment.

Many of the conclusions drawn above for the AAS detector are also valid for the ICP detector. For the ICP, the continuous feed of aqueous carrier solution prevents salt formation at the nebulizer nozzle face. Such encrustation may occur if air is introduced intermittently as it is during conventional operation. Using FIA, constant conditions are also created for the carrier gas in the nebulizer. Furthermore, rinsing cycles can be implemented using a second, independent pump. The rinse solution can be added intermittently, either to the liquid carrier stream before it enters the nebulizer or directly into the nebulizer just behind the nozzle face. FIA clearly improves the overall performance of atomic spectroscopy detectors. The automation of selectivity enhancement and preconcentration techniques significantly decreases analysis time and increases precision.

4.7 ELECTROCHEMICAL DETECTION

Electrochemical detection is based on the transport of an electroactive species towards the surface of a sensor. The detector responds only to the species which are present in the absolute vicinity of the sensor surface. Thus, these species must be representative for each portion of analyte solution which passes by the sensor. As a consequence, the design of the detector cell and of the hydrodynamic system becomes more critical for electrochemical detectors than for photometric detectors. The latter category of detectors integrate properties characteristic for much larger portion of the analyte.

Are hydrodynamic systems such as FIA systems suitable for creating reproducible analyte transport to the sensor surface? Yes, indeed they are, provided that the flow can be kept relatively free from pulsations. Exposure times can be optimized and kept constant for both samples and standard solutions. The inherent properties of the analyte gradient in the FIA system can also be exploited. Selectivity can be improved by kinetic discrimination of the interfering side reactions that occur at the sensor surface. The

common denominator for all these improvements are that reproducible, dynamic conditions for analyte production and presentation to the detector can be created in the FIA system.

The configurations of electrochemical detection cells are almost as numerous as the number of papers describing electrochemical detection. Basically, any design falls into one of the following three categories: a) wall-embedded, b) cascade type and c) end-on sensors, see Fig. 4.4.
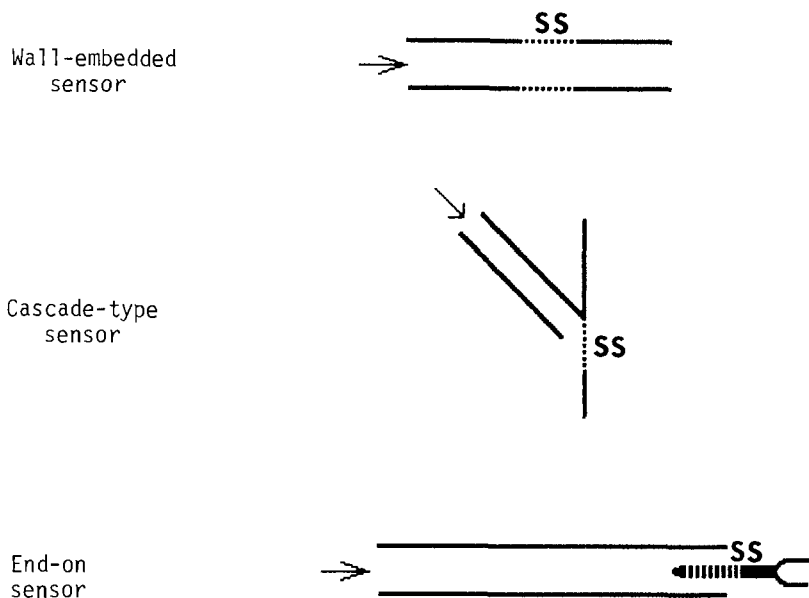
Fig. 4.4. The three basic configurations for electrochemical detectors. SS = sensing surface.

The wall-embedded sensor may be comprised of a cylinder inserted as part of the flow system or it may be comprised of one or several plates incorporated into the tubing wall. The cascade type sensor is exposed to the carrier stream either in a tangential or frontal manner. If a tangential flow is used several sensors can be positioned after each other in series. The end-on category most frequently entails wire-shaped sensors positioned in the center of the flowing stream so it is "piercing" the parabolic head of the sample plug. The positioning of reference and counter electrodes is less critical. In most cases they are placed after the sensing electrode in the flowing stream, either in the stream itself or in a waste reservoir.

The so-called walljet electrode is a hybrid of cascade and end-on electrochemical cell designs. The walljet has some potentially useful qualities for FIA. As mentioned earlier, the possible electrode and cell designs seem to be unlimited as long as the ohmic (IR) drop, between the different electrodes is matches the requirements of the electronics

involved. A large IR drop should be avoided since it may lead to a non-linear respon: of the sensor. How can a large IR drop be avoided? The magnitude of the IR drop governed by the distance between the electrodes, by the hydrodynamic conditions and I the electrolyte content in the carrier stream. So the first, obvious measure would be to u: a design where the distance between the electrodes is short. Then an inert electrolyte c: be added to the carrier solution. If voltammetry is applied a counter electrode can I implemented as an auxiliary, third electrode to carry electrical current thereby "protectin the reference electrode.

Which basic techniques can be utilized for FIA electrochemical detection? TI answer is straightforward: most current techniques can be applied when an appropriate c design is made. Table 4.1 lists the FIA electrochemical techniques and summarizes ti fundamental principles.

TABLE 4.1

Electrochemical techniques used in FIA.

Technique	Principle
Amperometry	Measurement of current at constant potential
Chronopotentiometry Potentiometric stripping analysis (PSA)	Constant current with measurement of potential with respect to time
Conductimetry	Inert electrodes with measurement of conductance
Potentiometry Ion selective electrodes Redox electrodes Ion-selective field effect transistors (ISFETs)	Measurement of potential
Voltammetry Polarography Anodic or cathodic stripping	Measurement of current at varying potential

Why do so many cell designs and electrode arrangements exist? The probable answer is that electrochemistry is a popular research topic. University budgets very often preclude investment in a commercial electrochemical detector, since these detectors are relatively inexpensive to make. A researcher can devote several months to design a detector with comparable capabilities. The research leads to improvements and the results are published. No other detection principle presents so many different versions and possibilities for the analysis of ascorbic acid and L-Dopa.

A survey of all FIA papers in which electrochemical detection is used would be very lengthy. The reader is referred to the bibliography index. However, ion selective electrodes deserve some further comments. The first FIA paper describing the use of ion selective electrodes was published in 1976 (8). The electrode was tilted 45° and the carrier stream was allowed to flow freely downwards and across the circular sensing surface of the electrode (cascade type). The reference electrode was placed in the outlet reservoir, see Fig. 4.5.
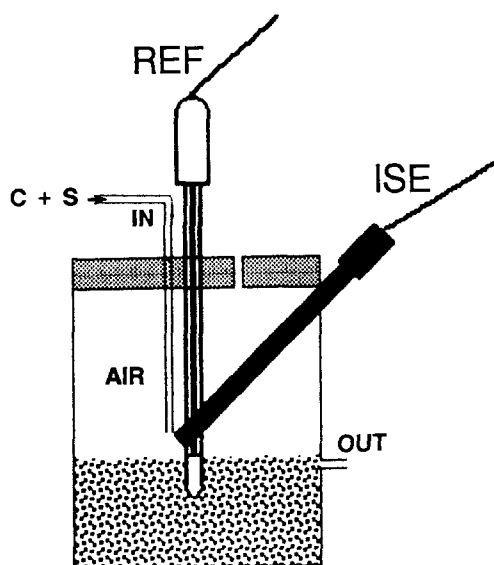
Fig. 4.5. First ion selective electrode detector system for FIA. C = carrier, S = sample, REF = reference electrode, ISE = ion-selective electrode.

Using this experiment set-up, the flow pattern at the surface of the ion selective electrode has a tendency to fluctuate. To avoid this fluctuation, the electrode can be immersed in liquid paraffin and a groove can be made in the paraffin film. The outlet stream from the FIA system is directed along the vertically positioned groove. Alternatively, a piece of coarse filter paper can be placed on the surface of the electrode.

In this way the delivered liquid is evenly distributed across the entire sensing area. Yet another possibility to avoid irregularities in the carrier/sample stream is to position the electrode vertically and to direct the stream frontally onto the center of the circular surface.

Ion selective electrodes respond rapidly and reproducibly in an FIA system. They seem to exhibit nearly Nernstian behavior in spite of non-steady state conditions (8). Selectivity coefficients obtained in this dynamic mode are often improved in favor of the main ion. In other words, the electrode is not responding as rapidly to changes in interfering ion concentrations as it to changes in the main ion concentrations (kinetic discrimination), see Fig. 4.6.

RESPONSE

FIA READING TIME   BATCH READING TIME

———————— MAIN ION

– – – – – – – INTERFERING ION
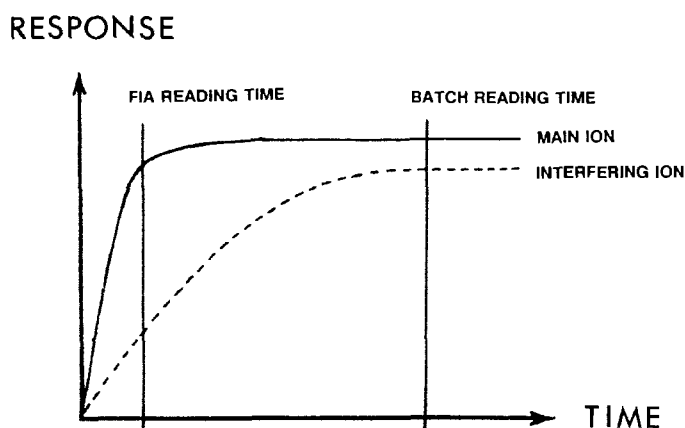
TIME

Fig. 4.6. Possible kinetic discrimination in electrochemical detectors.

The exactly defined exposure time established in any FIA system with electrochemical detection eliminates the arbitrary time delays which are difficult to avoid for manual measurements. The efficiency by which a sample with a high concentration is removed from the sensing area of the FIA detector is much higher in comparison with corresponding process in manually measured samples with stirring. Samples with high and low concentrations can therefore be introduced alternately in the FIA system with a maintained sample throughput rate.

4.8 OTHER DETECTORS

Virtually any detector which can accommodate a flow cell or accept a flowing stream can be used as an FIA detector. In many situations, the irreproducible handling of samples, reagents and timing lead to the observed imprecision of the detectors.

A few of the other notable FIA detector combinations are FIA-Fourier Transform Infrared, (FTIR); FIA-Raman, and FIA-Mass Spectrometry, (FIA-MS). The reasons for using these detector is that they are specific detectors. In FIA, the primary purpose is to improve reproducibility and to present to the detector a measurable form of the analyte.

In the case of FTIR, the absorption exhibited by water has limited its use in all flowing systems, HPLC included. However, the advent of the circle cell and the newly designed micro circle cell make aqueous flow through measurements using FTIR possible (9). Fig. 4.7 shows a circle cell. It has been shown that with the rapid scanning capabilities of FTIR, good vibrational spectra can be obtained in a continuous flow mode. This type of work has been extended using FIA to improve the detection limits and to allow chemistry, in particular derivatization, to be carried out before the FTIR measurements. This method seems to be especially useful for pharmaceutical analysis.

One application of FIA-FTIR is the determination of dioctyl sulfosuccinate in pharmaceutical samples. This particular application required a stopped-flow procedure because a slower and less sensitive detector, triglycine sulfate detector, was used. Detection limits were 0.6% w/v. The determination of succinylcholine chloride and bethanechol chloride were accomplished with the circle cell and continuous flow FIA. A linear range of 0.5 to 50 parts per thousand with a detection limit of 40 μg was observed.
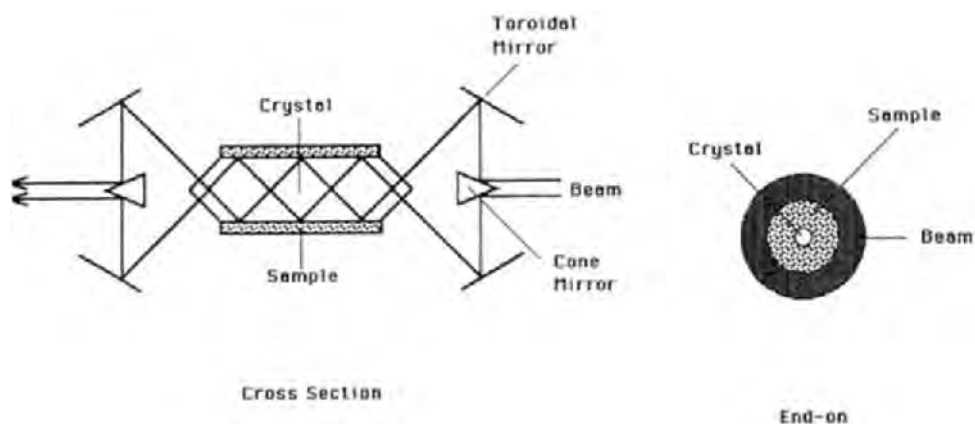
Fig. 4.7. FIA circle cell interface.

A most intriguing use of FIA with Raman spectroscopy is to reproducibly produce the analyte coating on silver sol microspheres (10). The silver coated spheres are then capable of exhibiting surface enhanced Raman. The increase in detection limit is substantial. The usual difficulty is to reproducibly produce a uniform coating of analyte on

the surface. This coating will give rise to the surface enhancements. By using FIA this difficulty can be overcome.

The connection of FIA to mass spectrometry provides the analyst with a very specific detection scheme. This combination can be used to determine the optimized conditions for a reaction (e.g. hydride generation) or to observe the actual species being produced by a reaction under varying conditions. A typical interface between FIA and mass spectrometry is shown in Fig. 4.8. To date, only dual phase gas diffusion has been coupled to MS. In this case a GC-MS was used. The dual phase gas diffusion manifold is connected to the MS by attaching the column inlet connection to the acceptor stream before the membrane. A jet separator is used in place of the membrane separator in order to optimize the elimination of the majority of the acceptor stream gas.

HEWLETT PACKARD 5993
QUADRUPOLE
GC/MS

FROM INJ. PORT   MS
                        INLET

OUTLET   DUAL PHASE INLET
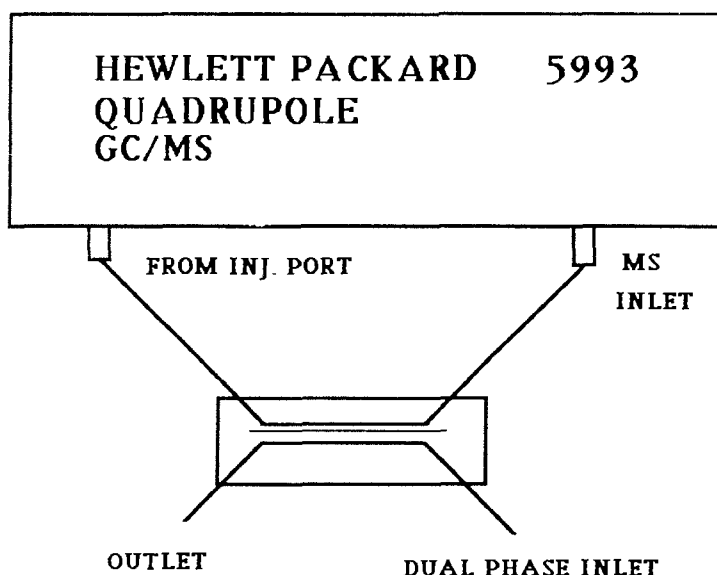
Fig. 4.8. Interface of an FIA to a mass spectrometer.

From a quantitative standpoint the combination of FIA-MS can be very useful for low level detection. The FIA system increases the precision of the sample introduction and the mass spectrometer is very sensitive. This combination makes detection of attogram $(10^{-18}$ g) quantities possible.

REFERENCES

1. J. Ruzicka and E.H. Hansen, Anal. Chim. Acta, 161 (1984) 1. [456]

2. K. Fukamachi, Anal. Chim. Acta, 119 (1980) 383. [98]

3. A.S. Attiyat and G.D. Christian, Anal. Chem., 56 (1982) 439. [381]

4. O. Astrom, Anal. Chem., 54 (1982) 190. [175]

5. P.N. Vijan and G.R. Wood, At. Absorption Newsl., 13 (1974) 33. [-]

6. M. Yamamoto, M. Yasuda and Y. Yamamoto, Anal. Chem., 57 (1985) 1382. [565]

7. Z. Fang, S. Xu, X. Wang and S. Zhang, Anal. Chim. Acta, 179 (1986) 325. [743]

8. J. Ruzicka, E.H. Hansen and E.A.G. Zagatto, Anal. Chim. Acta, 88 (1977) 1. [8]

9. B. Miller, N.D. Danielson and J.E. Katon, Appl. Spect., 42 (1988) 401. [-]

10. A. Berthod, J.J. Laserna and J.D. Winefordner, Appl. Spect., 41 (1987) 1137. [-]

CHAPTER 5

MODES OF OPERATION

5.1 INTRODUCTION

This chapter is devoted to describing the normal and special modes of operation used in FIA systems. It is the objective of this Chapter to explain not only the modes of operation, but to examine the reasons for their development and when they should be used. Sometimes it would seem that for every method there are an infinite number of ways of designing the manifold. But the main objective behind any design is to make sure it meets the analytical needs for the desired determination. Usually the simpler the design, the better!

5.2 BASIC MODES

What is needed to make an FIA system? A pump to create the continuous flow, an injector to introduce sample, some tubing and connectors to mix the reagent and sample, and a detector which responds to the analyte of interest. When these components are put together, as in Fig. 5.1, a single line FIA system has been created.
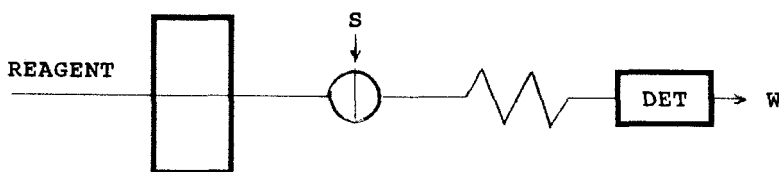
Fig. 5.1. Single line FIA manifold. S = sample, DET = detector, W = waste.

So now let us ask, when would a single line system be the optimal design for a method? Basically, when no additional chemistry is occurring and limited dispersion is desirable such as the case of a specific detector (i.e. ion selective electrodes, atomic absorption). The determination of potassium ion in electrolyte solutions by flame photometry is a good example. For this application the FIA system is used only as a transport system for the sample up to the detector. No dilution or chemical reaction takes place.

What would happen when some chemistry must occur? For example, in colorimetry the sample and reagent must react to form the reaction product which is eventually measured. Can a single line system be used? Maybe! To answer this question it is first necessary to think about the mixing process inside the tubing. Fig. 5.2a shows the sample plug at the point of sample injection. At this point no physical mixing and no sample/reagent reaction has occurred. In Fig. 5.2b, the sample zone begins to move down the tube and physical mixing now begins. Note that both sample and reagent have begun to disperse into each other at both ends of the sample zone. This physical mixing will occur continuously as the sample zone moves down the tube. Eventually, if enough tubing is used, the sample zone and reagent will be mixed, see Fig. 5.2c. When the RS plug in Fig. 5.2c passes through the detector a normal FIA peak will be observed.
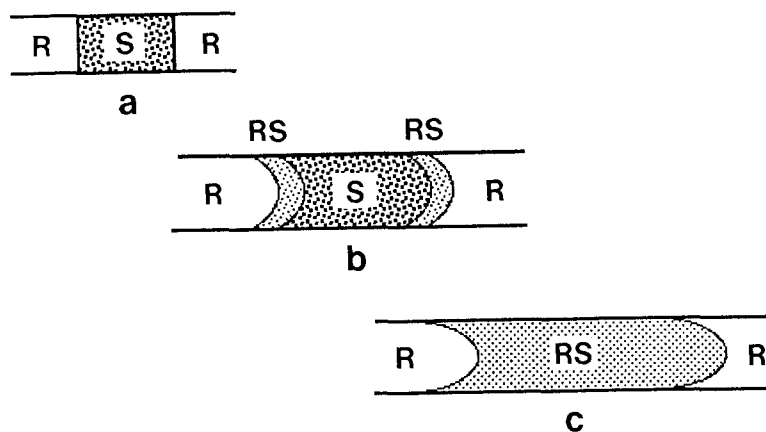
Fig. 5.2. Depiction of the mixing process in a single line FIA system a) sample plug at point of injection, b) sample further down the tube where mixing begins, and c) complete mixing of sample and reagent. R = reagent, S = sample, RS = reaction product sensed by the detector.

Is increasing the tubing length to allow for complete mixing in a single line system the best approach? Not really, because the extra time needed to accomplish this and the additional dispersion are without merit.

There is an additional critical assumption in the single line approach namely that the reagent concentration is sufficiently high to react with all the analyte in the sample. Is this a valid assumption? Not necessarily, and in most cases no! The observed double peak, see Fig. 5.3, is indicative of either incomplete mixing or insufficient reagent concentration.
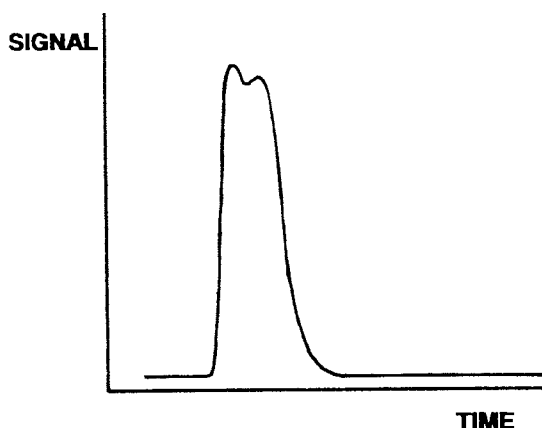
Fig. 5.3. Double peak caused by incomplete conversion of S to RS due to insufficient R concentration.

How can these problems be avoided? The recommendation is that at least a two line system be used, see Fig. 5.4.
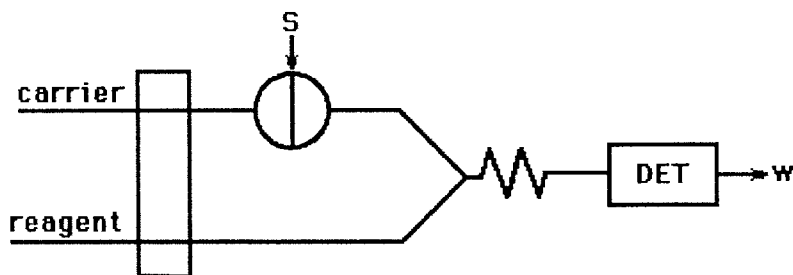
Fig. 5.4. Two line FIA manifold provides for a constant reagent concentration across the entire sample zone. S = sample, DET = detector, W = waste.

With this design the reagent and sample plug meet at the tubing connector, see Fig. 5.5a. At this point, the sample would be placed on top of the reagent (Fig. 5.5b). As this mixture passes through the mixing coil, physical equilibrium is approached. Eventually, the entire sample zone has been mixed with the reagent (Fig. 5.5c). Therefore, there is no shortage of reagent in the center plug.
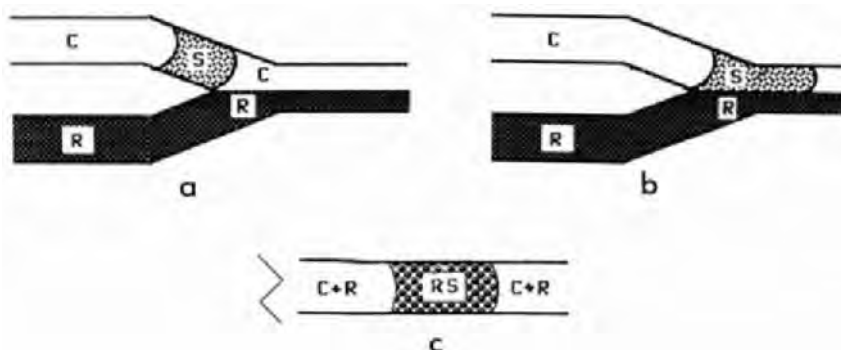
Fig. 5.5. Depiction of the introduction of sample and reagent to each other in a two line manifold. a) sample and reagent meet, b) sample and reagent begin to mix, and c) reaction is complete. C = carrier, R = reagent, S = sample, RS = reaction product sensed by the detector.

It should be mentioned that it would still be possible to have such a high concentration of analyte that the reagent concentration is insufficient to react totally with that analyte. The obvious indication of this problem is that changes in sample concentration do not change the observed peak heights. This problem can be handled by using the manifold for dilution or by using a gradient technique (Chapter 7).

In conclusion, the basic modes are single line and multiple line systems where the reagent and/or carrier streams are continuously pumped. The sample is injected into the system in such a way as to ensure complete physical mixing and adequate reagent supply.

5.3   SPECIAL MODES, ONE PUMP

There are four special modes in which only one pump is required, namely merging zones, reverse flow, split loop injection, and stopped-flow. In each case, there are specific practical reasons for these design modifications. The first of these practical reasons is if the cost of the reagent is significant. Therefore, the design of the manifold must be made so that reagent consumption is minimized. How can this requirement be met? The immediate thought is to immobilize the reagent onto a packing material or membrane. This has been demonstrated as being effective. However, the disadvantages are that the immobilization requires lengthy preparation and may cause loss of reactivity. Such is often the case with enzymes. A further disadvantage is that the immobilized reagent can be poisoned by certain samples. Is there a way to conserve reagent using a special FIA mode or manifold? One common approach is a technique called merging zones.

The principle behind merging zones is shown in Fig. 5.6a-c. In two separate tubes, one reagent and one sample plug are injected, see Fig. 5.6a. These plugs move down the tube to the intersection of the tubes where they begin to merge, see Fig. 5.6b. Finally, S and R mix and react and the detected species, RS, which is surrounded by C, is brought to the detector, see Fig. 5.6c.
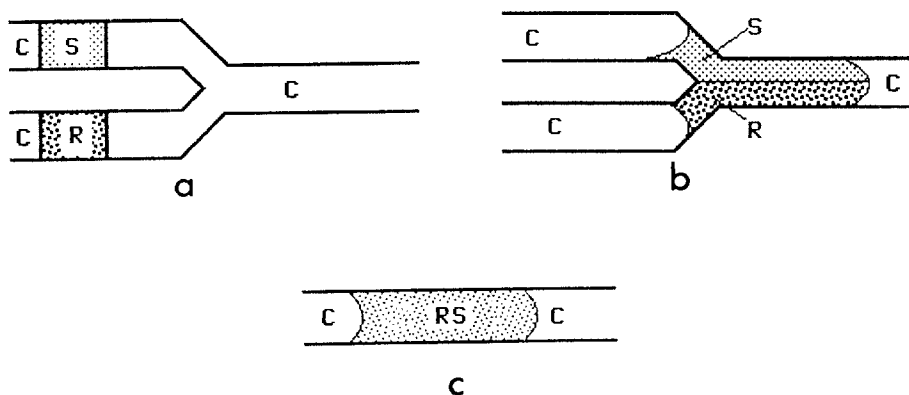
Fig. 5.6. Depiction of the merging zone principle. a) reagent and sample plug before mixing, b) reagent and sample plug at point of merge, and c) reaction product, RS, bracketed by carrier, C. R = reagent, S = sample.

Complete mixing is created by the coil which is placed after the connector. This design is the same as the basic multiple line system, except that the reagent is not continuously pumped but introduced as a discrete zone. The timing must be perfect and the reagent volume slightly larger than the sample volume in order to ensure effective and reproducible results. One way of accomplishing merging zones is shown in Fig. 5.7.
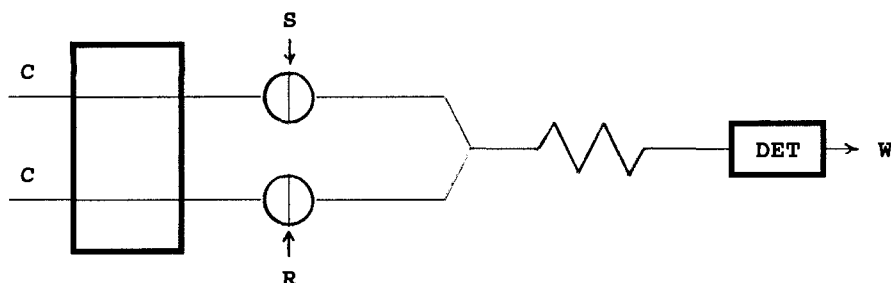
Fig. 5.7. Manifold for merging zones. C = carrier, S = sample, R = reagent, DET = detector, W = waste.

A second approach is to utilize reverse flow injection analysis (rFIA). In rFIA the reagent is injected and the sample and carrier are continuously pumped. The assumption when using this method is that this application is only practical when the amount of sample available is large. This was the case with an FIA system used on board a ship to examine ocean water chemistry (1). The design of such a system is shown in Fig. 5.8.
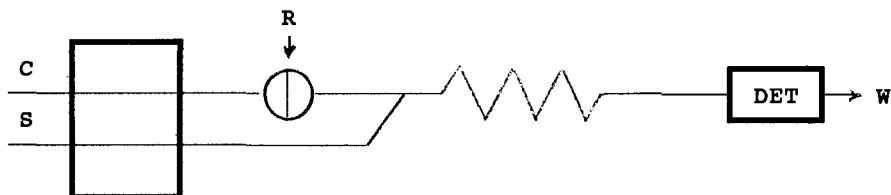
Fig. 5.8. Manifold for reverse FIA. C = carrier, S = sample, R = reagent, DET = detector, W = waste.

A third option is split loop injection. This technique is a combination of injection and the chasing zones principle (Chapter 7). The hardware set-up is shown in Fig. 5.9. The principle is that the normal sample loop is divided into two sections (1 and 2) which share a common waste outlet. With the valve in the fill position, see Fig. 5.9a, both reagent and sample solutions are simultaneously aspirated by the pump, filling tubing sections 1 and 2, respectively. When the valve turned, see Fig. 5.9b, sections 1 and 2 are inserted into the carrier stream and will be brought to the manifold of the FIA system. Thus, both zones proceed down the tubing with the sample chasing the reagent (the opposite situation is also acceptable). The dimensions of the manifold determine the amount of dispersion for the two zones and therefore, the amount of overlap before the detector is reached.
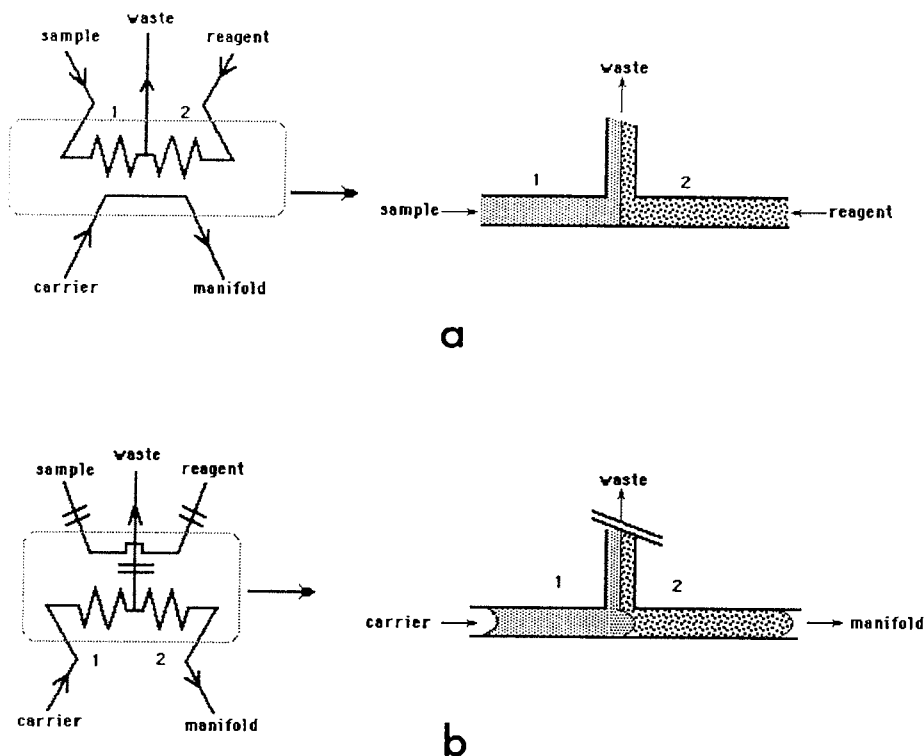
Fig. 5.9. Depiction of the split loop injection technique. For details, see text.

An additional modification of this approach is shown in Fig. 5.10. Both sample and reagent are simultaneously aspirated or pumped into the tubing section labeled as 1 (Fig. 5.10a). The volume ratio between sample and reagent solutions is determined by the flow rates F_r and F_s. The injector is turned and the premixed section of solution is inserted into the carrier stream. The amount of reagent solution is controlled by the flow rate ratio. Other variations based upon this approach are also possible.
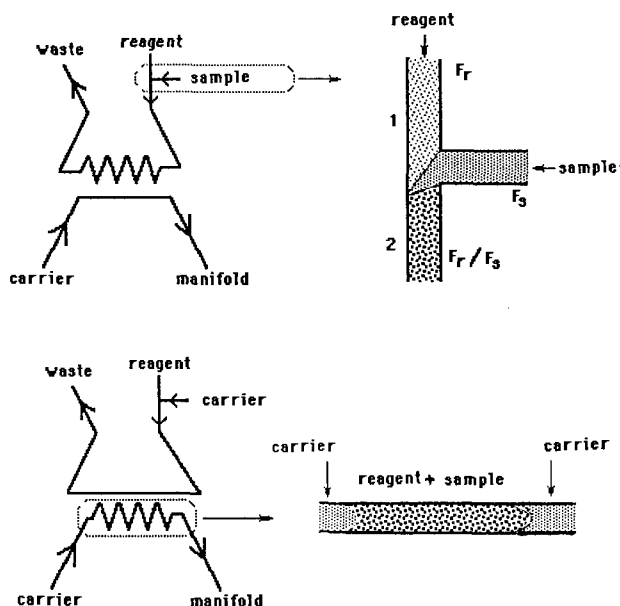
Fig. 5.10. Modified split loop injection technique. For details, see text.

With the split loop approach the amount of reagent used is dependent on the tubing diameter and the flow rate. By cleverly manipulating these parameters, conservation of the reagent can be achieved. Whether split loop injection is better than the merging zone approach is difficult to state. However, the decision may be made based on the availability of equipment. If one pump and one injector are available, then split loop injection would be the technique to use. If one pump and two injectors are available merging zones is possible. Two pumps and one injector would make the merging zone - intermittent pumping technique discussed in Chapter 5.4, possible.

One final mode, which has nothing to do with reagent conservation, should be briefly mentioned in this section, stopped flow. It is desirable to stop both carrier and reagent streams in the detector cell or before the cell. Stopping the sample zone in the cell allows for the observation of the degree of reaction completion. Stopping the sample

zone before the cell increases residence time and therefore reaction time without adding dispersion. In Fig. 5.11 three situations are depicted. The "a" line represents a situation in which the reaction is not complete at the time when the flow is stopped. This suggests that the reaction coils should be lengthened or the flow rate decreased in order to increase reaction time. There is one caution at this point. It should be determined whether the observed increase is due to the analyte/reagent reaction or some photoinduced reaction. This can be accomplished in part by stopping the flow before the sample zone is in the cell and observing any change in signal due to the reagent and carrier. If no change in signal is observed, no photoinduced reaction occurs. The "b" line shows that the reaction is complete. The "c" line suggests that something in the system is decreasing the concentration of RS, the detected species. This could be due to a chemical side reaction or merely decomposition of the reaction product, RS.

Stopped-flow has many other possibilities which will be discussed in Chapter 7.
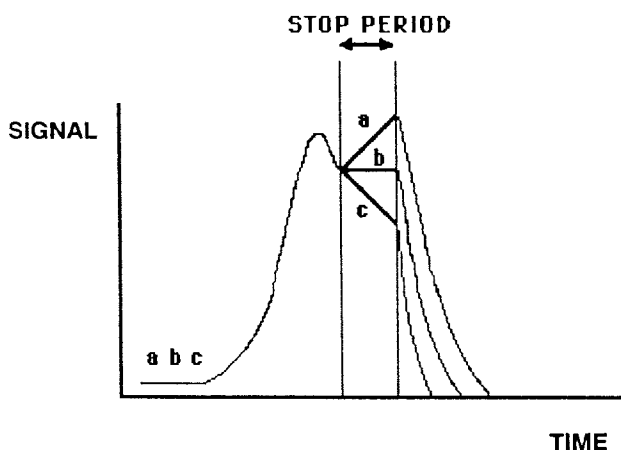
Fig. 5.11. Signal from a stopped flow system.   a) reaction not complete or additional reactions occurring, b) reaction complete, and c) decomposition or quenching occurring.

5.4   SPECIAL MODES, TWO PUMPS

Special modes that use two pumps can, for the most part, can categorized as intermittent pumping techniques. The general categories include sample and reagent conservation, washing and conditioning, injection, and dilution. At some step in a two

pump method, one of the pumps is off while the other remains on or is turned on. If both are turned off simultaneously, then this is the stopped-flow mode. The two separate pump systems are programmed independently to produce the desired pumping cycle.

The first case is where the amount of sample is small. How can two pumps be used to conserve sample? If one pump is used exclusively to aspirate sample, it can be turned off to save sample after the sample loop has been filled. Therefore, the other pump is used to control the continuous flow of the reagent and carrier streams. Such a system is shown in Fig. 5.12. The important point is to minimize the amount of tubing from the sample to the sample loop of the injector. Enough time must be allotted to not only fill the loop but to ensure no sample carry-over. If this technique is used in conjuction with an automated sampler, the tubing and sample loop can be washed out with water. The same thing can be done without a sampler if an additional valve is used.
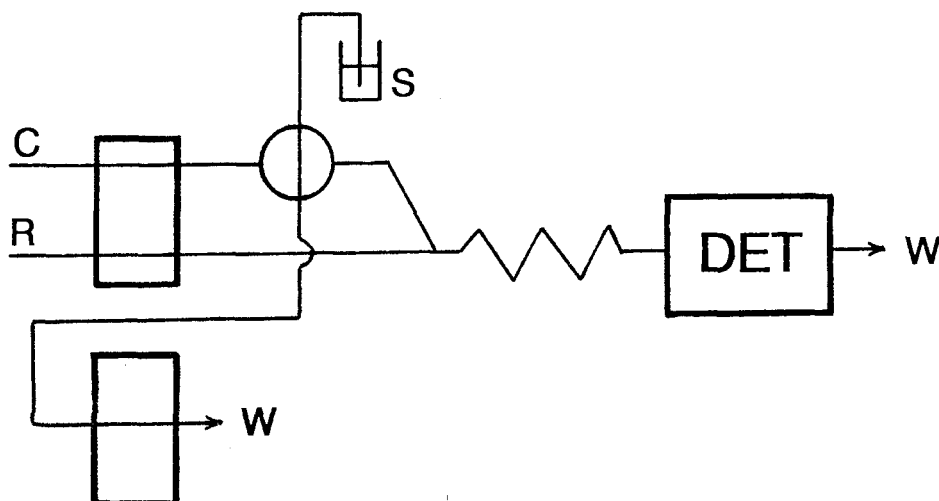
Fig. 5.12. Diagram of an intermittent pumping manifold. C = carrier, S = sample, R = reagent, DET = detector, W = waste.

The next situation is where the reagent is expensive and needs to be conserved. How can a multiple line system be modified in order to provide for reagent conservation using two pumps? An option is to incorporate two pumps and have the reagent intermittently pumped into the mainstream at the time that the sample zone passes the connection point. This is another form of merging zones, see Fig. 5.6. The carrier is continuously flowing, but the reagent line is activated only when the sample zone is approaching the connection between sample and reagent. The reagent is then added to

the sample zone much like butter to bread. This buttering approach will conserve reagent and can be optimized in terms of time to improve the signal.

From time to time it becomes necessary to wash and/or recondition the system. This can be accomplished with intermittent pumping. Many times an additional valve or a two channel valve is necessary. For example, an FIA system which has an ion exchange resin in a packed bed reactor would have to have a reconditioning cycle for the ion exchange resin. If the packed bed reactor is placed in the valve where a sample loop is normally located, the valve and intermittent pumping can be coupled to produce a rapid system. Fig. 5.13 shows this system.
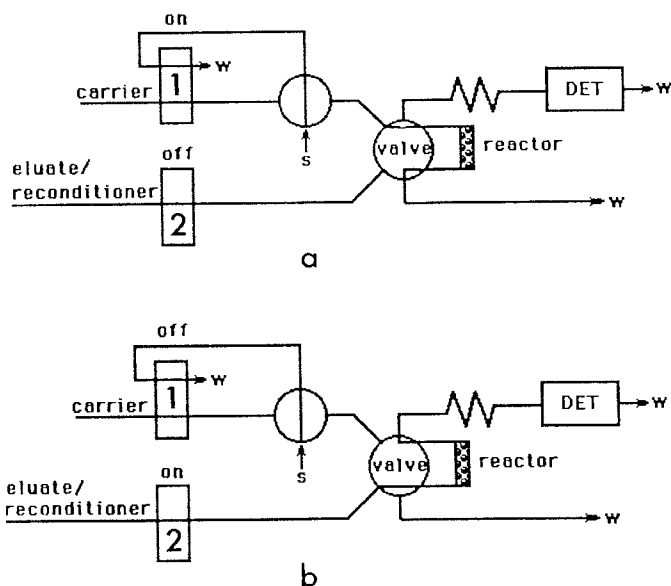
Fig. 5.13. Manifold design for regeneration and elution of a column or packed bed.
S = sample, DET = detector, W = waste. a) preconcentration step, and b) elution step.

The sample is aspirated and the carrier and reagents are continuously pumped by pump one. The analyte ions are captured on the ion exchange resin. The valve is turned as pump one stops and pump two starts. The reverse flow through the column reduces band broadening. The eluting solution also reconditions the column. After the peak is detected the valve turns as pump two stops and pump one starts. A new sample can now be injected.

Concern has been expressed over the long term reliability of injectors. For the most part, this concern is unwarranted. But is there an alternative way to inject a sample without an injection valve? With two pumps it is possible to inject a definite volume of

solution, reagent or sample, into an FIA system. Fig. 5.14 is the schematic diagram of such a system. The point of interest is the area between points a and b. When pump 1 is running and pump 2 is off, the FIA system is a normal one reagent system but without an injector. If pump 1 is turned off, and the waste line is fed back through the pump in order to block the flow path, the tubing segment between a and b can be filled by starting up pump 2. When pump 2 is stopped and pump 1 is started, the trapped segment of sample is injected into the system. This approach is called hydrodynamic injection.

At first glance it, would seem that this approach to sample injection would be less precise. However, hydrodynamic injection is very precise since the basic principles of FIA are not violated. The tubing segment does not change size, therefore the volume is constant. Pump on/pump off timing is critical. The fill cycle for the tubing segment is governed by its volume.
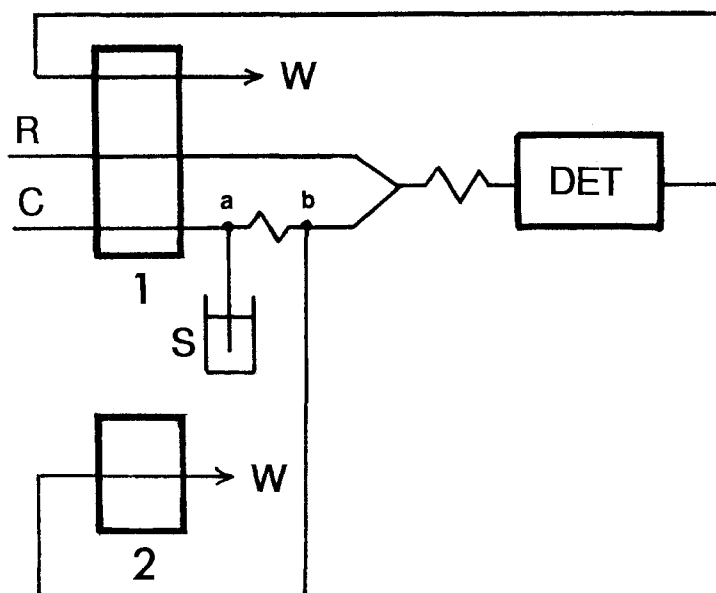
Fig. 5.14. Manifold design for sample injection with two pumps, hydrodynamic injection. C = carrier, R = reagent, S = sample, DET = detector, W = waste. The "injection loop" is situated between a and b.

There are other manifold designs that can be used for hydrodynamic injection. However, in all hydrodynamic injection manifolds, careful attention to flow rates is critical.

5.5   DILUTION MODES

As a rule, the standard multiple line manifolds will have a dispersion coefficient, D, of 2 - 6. How can dispersion coefficients of between 10 - 30, 20 - 500, and 50 - 7000 be achieved without using a gradient chamber? Dilutions of 10 - 30 can be achieved most conveniently by using the manifold shown in Fig. 5.15.
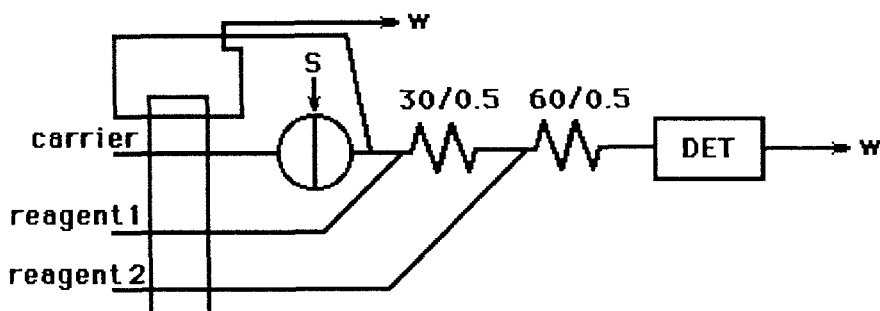
Fig. 5.15. Manifold design for sample splitting. DET = detector, S = sample, W = waste. 30/0.5 and 60/0.5 denote a 30 cm and a 60 cm long coil, respectively, both with an i.d. of 0.5 mm.

The sample splitting method shown is accomplished by drawing off a portion of the injected sample. In this case the flow rate of the carrier stream is 0.8 ml/min while the waste is withdrawn at 0.6 ml/min. Table 5.1 presents the observed dispersion coefficients range from 7 - 32 depending on the flow rates and the injection volume. After the dilution a normal manifold design is used for chemistry, in this case a two reagent system.

TABLE 5.1

Sample splitting data for dispersion coefficient, precision, and residence time with different injection volumes.

C ml/min*	Waste ml/min*	Injection volume μl	D	r.s.d. %	Residence time sec/sample
0.8	0.6	40	32	1.3	35
0.8	0.6	100	18	0.7	45
0.8	0.6	200	13	<0.5	55
1.2	0.8	40	19	1.0	30
1.2	0.8	100	9	1.1	40
1.2	0.8	200	7	<0.5	50

* All flow rates are nominal. The actual flow rates are slightly lower throughout.

For dispersion coefficients ranging from 20 - 500, the technique of zone sampling is appropriate. The manifold for zone sampling is shown in Fig. 5.16.
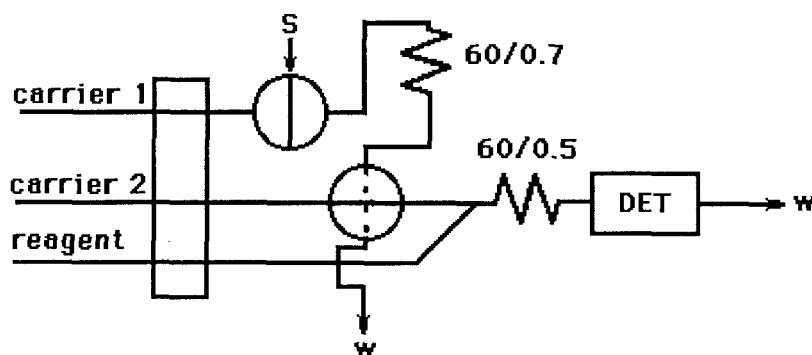
Fig. 5.16. Manifold design for zone sampling. S = sample, DET = detector, W = waste. 60/0.7 and 60/0.5 denote 60 cm long coils, the first one with an i.d. of 0.7 mm and the second one with an i.d. of 0.5 mm.

The sample, in this case 40 μl, is injected into the carrier stream, Fig. 5.17a, and is dispersed by, for instance, a 60/0.7 mixing coil before it enters the second injection loop. The resulting axial concentration relationship between C and S is illustrated in Fig. 5.17b. When the injector returns to the fill position, a portion of the dispersed sample is reinjected into a second carrier stream, Fig. 5.17c. Now, any desired chemistry can be induced by the usual addition of reagents. The two injection volumes are hereinafter called V_1 and V_2.
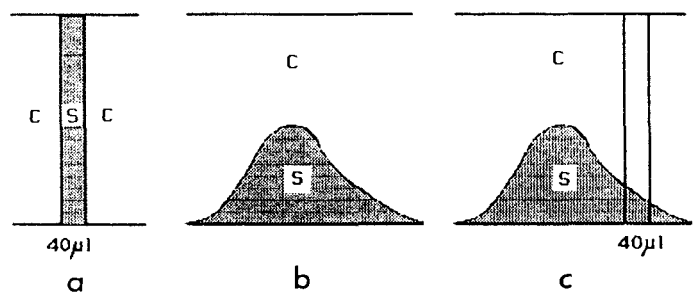
Fig. 5.17. Depiction of axial sample dispersion under zone sampling conditions. C = carrier, S = sample. For details, see text.

Table 5.2 shows the effect of variations the two injection volumes V_1 and V_2 while Table 5.3 shows the effects of variations in injection time.

TABLE 5.2

Variations in injection volumes using zone sampling. Flow rates: C_1 = 1.1 ml/min, C_2 = 0.7 ml/min, R = 1.8 ml/min. Injection time = 14 sec.

Injection volumes μl		Dispersion coefficient	r.s.d. %
V_1	V_2		
40	40	35	2.8
40	100	29	1.8
40	200	27	1.2
100	100	18	0.8
200	40	22	5.3

As can be seen in Table 5.2 only a small change of D can be achieved by varying the injection volumes when using the zone sampling technique.

Table 5.3 shows the dramatic effect that variation of the injection time has on the dispersion coefficient. The only negative effect is that as the dilution increases the precision attributed to the dilution step decreases. However, it should be realized that if a 5400 fold dilution were to be performed manually, the overall error due to the volumetric glassware could create comparable r.s.d. values.

TABLE 5.3

Variations in injection time using zone sampling. Flow rates: C_1 = 1.9 ml/min, C_2 = 1.8 ml/min, R = 1.8 ml/min. Injection volumes: V_1 = V_2 = 40 μl

Injection time sec	D	r.s.d. %
8	23	1.2
12	78	1.2
15	410	2.1
20	5400	4.6

The two techniques of sample splitting and zone sampling can also be combined. This is shown in Fig. 5.18. Sample splitting is accomplished first and then zone sampling. At carrier flow rates of 1.1 ml/min, reagent flow rates of 1.9 and 0.7 ml/min for R_1 and R_2, respectively, and split flow rates to waste of 0.7 ml/min, a 15, 25, and 29 sec injection time produced 190, 1200, and 5000 dispersion coefficient values and 3.7, 1.2 and 5.4 % r.s.d. values, respectively.
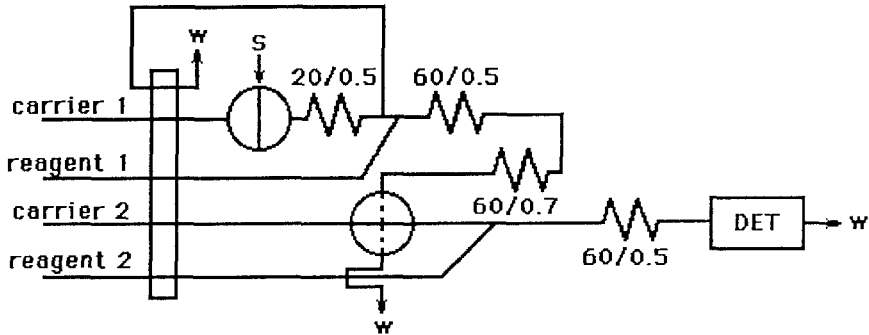
Fig. 5.18. Manifold combining sample splitting and zone sampling for a two reagent system. S = sample, DET = detector, W = waste. 20/0.5 denotes a 20 cm long coil with an i.d. of 0.5 mm (dimensions denoted correspondingly for the other coils).

For dispersion coefficients of 50 - 7000, the reverse set up would be desirable. The manifold for this type of operation is shown in Fig. 5.19 while Table 5.4 displays some typical results for this type of system.
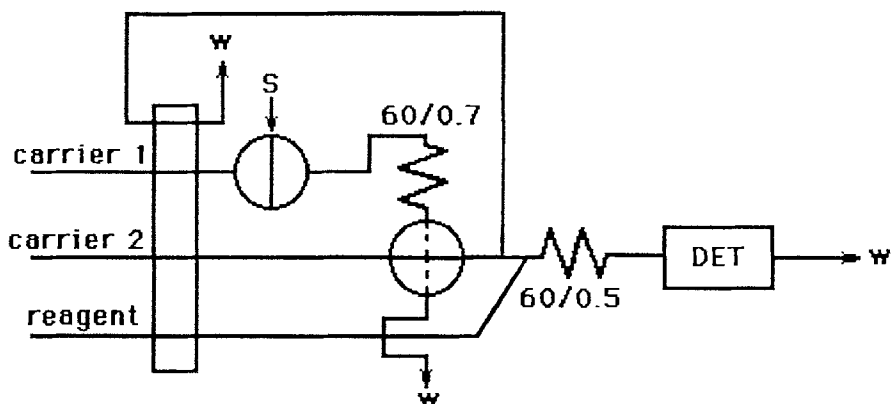
Fig. 5.19. Manifold combining zone sampling and sample splitting. S = sample, DET = detector, W = waste. 60/0.7 and 60/0.5 denote 60 cm long coils with an i.d. of 0.7 and 0.5 mm, respectively.

TABLE 5.4

Zone sampling followed by sample splitting. Flow rates: C_1 = 1.9 ml/min, R = 1.8 ml/min. Injection volumes: V_1 = V_2 = 40 μl.

C_2 ml/min	Waste flow ml/min	Injection time sec.	D	r.s.d. %
1.8	0.7	8	50	2.1
1.1	0.7	12	390	2.7
0.7	0.6	15	1360	8.4
0.8	0.6	18	6900	3.6

As can be seen in Table 5.4, when the flow rates of C_2 and the split flow to waste are too close, large r.s.d. values result. However, the last entry in the Table dramatically shows how the combined techniques can be used to obtain large dilutions with acceptable r.s.d. values.

5.6   REVERSE FLOW, FLOW INJECTION ANALYSIS.

The last special case to be mentioned is reverse flow, flow injection analysis, which is in its infancy. In this technique the flow direction of the flow streams are reversed from time to time. The idea is that the flow reversals will maximize residence time or contact time with a reactor or separator while minimizing dispersion. Only a few limited examples have been given in the literature at this time, however the technique should be kept in mind as one to watch.

One example would be an in-line filter manifold, see Fig. 5.20. In this application, the reverse flow serves the purpose of backflushing the in-line filter. The sample is injected and merged with the reagent passing through the mixing coil. Next, the sample is passed through an in-line filter. The sample, then, enters the detector. Before the sample starts to leave the detector, pump 1 is stopped and pump 2 starts, thereby reversing the flow through the detector and the in-line filter. The back flushing of the filter, reconditions the filter for the next determination.
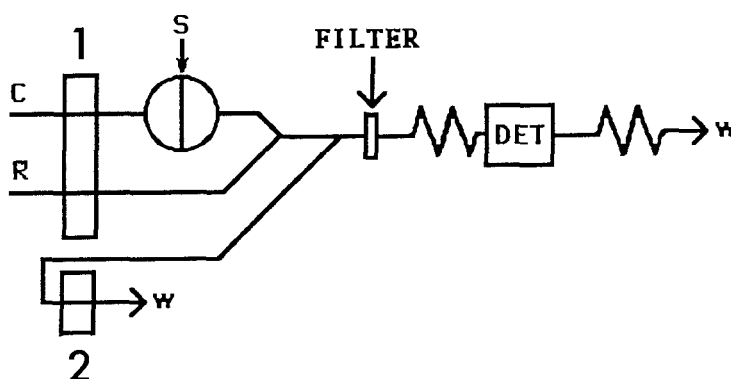
Fig. 5.20. Manifold for a reverse flow, flow injection analysis with an in-line filter.
C = carrier, R = reagent, S = sample, DET = detector, W = waste.

The observed peak is different from the normal FIA peak. Since the tailing section of the dispersed sample zone never passes through the detector, the detector response is symmetrical. What went in comes right back out! Other practical reasons for reverse flow FIA will undoubtedly be seen in the future.

REFERENCE

1.   J. Thomsen, K.S. Johnson and R.L. Petty, Anal. Chem., 55 (1983) 2378. [343]

CHAPTER 6

SELECTIVITY ENHANCEMENT, MATRIX MODIFICATION AND CONVERSION TECHNIQUES

6.1   INTRODUCTION

Selectivity is the ability of a method or instrument to specifically determine an analyte in the presence of a specified set of interferents. The method development process is an exercise in creating the desired selectivity, selectivity enhancement, to meet the stated method criteria. To achieve such enhancements, reagents, specific detectors, separations and kinetics have all been used. However, if the ultimate objective of the method is for rapid, automatic and continuous determinations an additional constraint, simplicity, is placed on the ideal method. Therefore, in many cases, chromatographic separations are too time consuming and selective detectors, such as ion selective electrodes and atomic spectroscopy, are much too limited in their applicability.

Selectivity enhancement in FIA is accomplished by using separation, matrix modification and conversion techniques alone or in combination. Matrix modification is the common term for a wide range of methods and techniques which can be performed in an FIA system. In Fig. 6.1, a simplified overview is presented.

Techniques based on "conversion" are very commonly applied in FIA systems and refer to analyte conversion. The analyte is converted to a species which can be more selectively sensed by the detector. For this purpose reagents can be added in confluent streams. Conversion can also take place in a small columns placed in the FIA system. The columns may contain immobilized reagents such as enzymes, catalysts or reducing/oxidizing agents. When several matrix modification and analyte conversion steps are performed in an FIA system the total selectivity enhancement accomplished will be the product of all the individual enhancements obtained in each step, i.e., the effect is multiplicative.

This chapter describes the most commonly used matrix modification techniques in FIA systems and also the special case of analyte conversion performed in in-line columns. The ultimate objective with these techniques is to accomplish selectivity enhancement which, most frequently, is identical with improving the signal to noise ratio. As a general rule, analyte conversion increases the signal while matrix modification reduces the noise produced by the detector.

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        ┌─────────────────────────────────────┐
        │  MATRIX MODIFICATION TECHNIQUES      │
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IN-LINE TRAPPING	ANALYTE TRANSPLANTATION	SOLUTION ADDITION
PARTICLES (using filters)	DIALYSIS A(L1) → A(L2)	DILUTION
IONS (using ion exchangers)	L/L EXTRACTION A(aq) → A(org)	BUFFERING (pH, ion activity, ion strength)
MOLECULES (using e.g. C_{18})	GAS DIFFUSION A(L1)→A(G)→A(L2)	MASKING
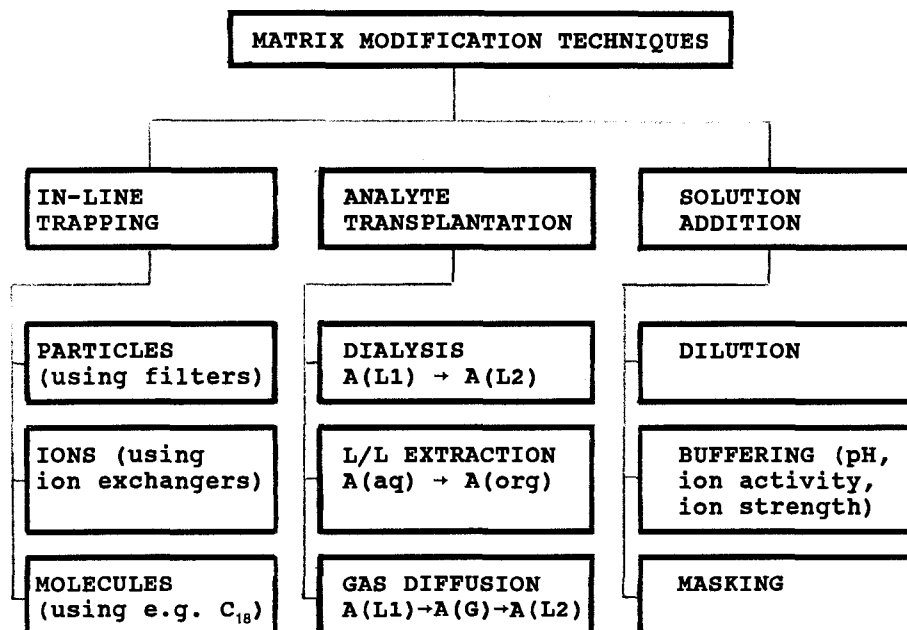
Fig. 6.1. Schematic presentation of matrix modification techniques. A(L1) = analyte in liquid phase 1, A(L2) = analyte in liquid phase 2, A(G) = gaseous analyte, C_{18} = C_{18} column.

6.2   LIQUID-LIQUID EXTRACTION

Manual, batch process liquid-liquid extraction is a tedious procedure and many efforts have consequently been made to bring about mechanization of this procedure. The classical batch approach needs no further explanation, while liquid-liquid extractions in continuous flow systems require some comments. Two immiscible phases are brought together in a narrow tube in a controlled manner so that defined segments of each phase are formed. The choice of tubing material, tubing dimensions and mixing geometry is critical for the final result. In the extraction tube, often coiled, the contact area between the two phases is large. The ratio between the tube area and the tube volume is also large. In the extraction vessel used in the batch method the corresponding ratios are much lower. Consequently, if the vessel material used to accommodate the two liquid phases in any way is involved in the analyte transfer process, this would have a larger influence in continuous flow extractions than in batch extractions.

The phase separator in a continuous flow system is designed to handle small volumes of either phase so that segments of the same phase are joined and completely separated from the segments of the other phase. The volume of the separator is often

small to prevent dilution of the sample or to prevent deterioration of the original concentration gradient of the sample.

The classical air-segmented analyzers had been used to perform liquid-liquid extraction long before the first FIA system was designed for the same purpose. A typical air-segmented system is shown in Fig. 6.2. The sample is pumped through the pump, segmented with air, mixed with an aqueous reagent and segmented with an organic phase. The air and the aqueous phase are removed before detection. The extraction coil material is, in most cases, glass. If a narrow-bore PTFE coil is used, a backpressure is easily built up due to the compressibility of the air segments.
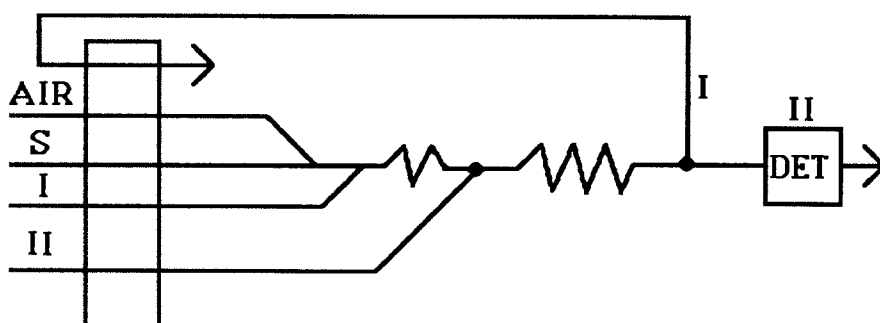
Fig. 6.2. Principle for liquid-liquid extraction in an air-segmented flow analysis system. S = sample (aqueous), I = aqueous reagent, II = organic phase, DET = detector.

The first continuous flow system for liquid-liquid extractions based on the flow injection principle was published in 1978 (1). The principle is shown in Fig. 6.3.
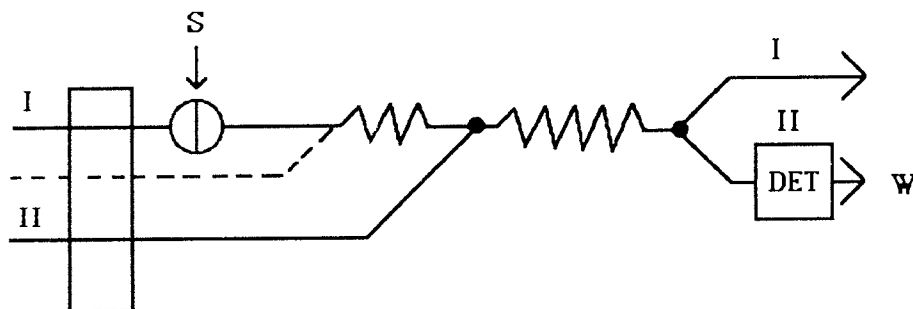
Fig. 6.3. Principle for liquid-liquid extraction based on flow injection. I = aqueous phase, II = organic phase, S = sample, DET = detector. Broken line denotes addition of an optional reagent to the aqueous phase.

A defined volume of the sample is injected into a carrier (or reagent) stream which is segmented with an immiscible solvent. Extraction takes place in a narrow-bore PTFE tube (i.d. typically 0.5 mm). The second phase, now containing the extracted analyte, is separated from the first phase and transported to the flow through detector. Common for both extraction manifolds (Figs. 6.2 and 6.3) is that the detector is implemented in-line.

In-line detection is, of course, the most elegant way of detection when a high degree of mechanization of the entire analytical procedure is desired. However, some in-line detectors are not easily added to an FIA system. The extraction manifolds can nevertheless be used as sample work-up systems. Fig. 6.4 shows the principle for a flow injection extraction system with off-line detection.
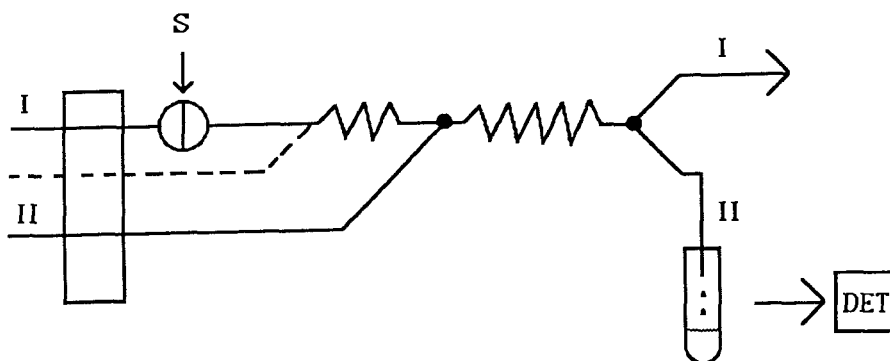
Fig. 6.4. Principle for liquid-liquid extraction in a flow injection system with off-line detection. I = aqueous phase, II = organic phase, S = sample, DET = detector.

An exact volume of sample is injected into the system. The entire extracted analyte is collected in a small vessel, diluted to a known volume and subsequently delivered to the detector. Using the manifold in Fig. 6.2 the sample collection step becomes more critical. A known volume of the sample can be pumped through the system and completely collected. Alternatively, a known volume of the sample is collected during the "steady-state period". What is meant by that?

In an air-segmented system the extraction does not reach steady state until a defined concentration level of analyte has been attained. Therefore, collection of the organic phase cannot be started until this level has been reached. Dilution to a known volume is not necessary in this case.

Analyte enrichment/preconcentration is one objective when performing liquid-liquid extractions. In the batch approach small volumes of organic phase can be combined with large volumes of the aqueous sample provided that the shaking is efficient. Could this be done in a continuous flow system? Yes, a certain variation of the phase volume ratio

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is possible but nevertheless limited to about 0.1 - 10 and only really practical in the range 0.25 - 4, i.e. a four fold difference in flow rate between the organic and aqueous phases is possible. Now, assume that a four fold enrichment is desired for an aqueous sample and that an AAS detector is to be used for analyte detection of organic phase. This detector requires a flow rate of about 3 - 5 ml/min if an organic carrier is utilized. This means that the flow rate of the aqueous phase must be at least in the range of 12 - 20 ml/min. Such flow rates are not practical. One way of solving the problem is to integrate two flow systems, one "low" flow rate system and one "high" flow rate system as shown in Fig. 6.5.
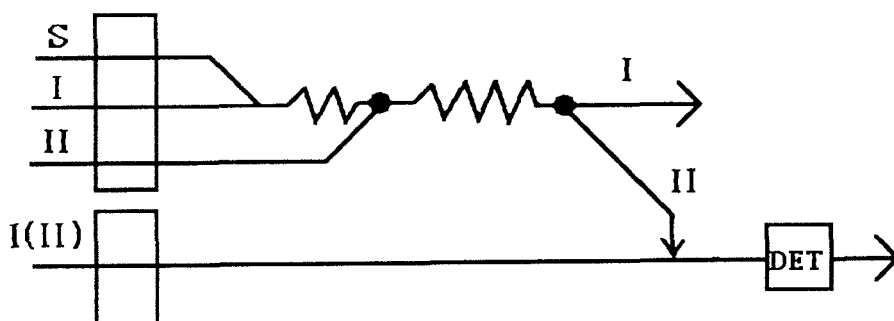
Fig. 6.5. Integration of two flow systems: the upper one is used for the extraction and the lower one is the detector feed system. S = sample, I = aqueous phase, II = organic phase, DET = detector.

This approach was applied by Nord and Karlberg (2) who extracted copper from an aqueous sample into methyl isobutyl ketone, MIBK, using APDC as a complexation reagent. The MIBK phase was separated and a portion was injected into an aqueous stream which was pumped into an atomic absorption spectrophotometer.

The manifold designs in Figs. 6.2 - 6.5 cover the majority of possibilities described in the literature but far from all the possibilities which do exist for liquid-liquid, continuous flow extractions. Back-extractions, for example, require additional manifold components to be added. Recirculation of one phase can be performed (3). In-line filters, ion exchange packed-bed reactors and solid chelate columns can also be implemented. Components to be used for flow injection extraction have to be selected with care, see Chapter 3.

Since small sample volumes are employed in FIA, a fundamental question is - what price has to be paid with respect to dilution or loss in sensitivity in comparison with batchwise treatment? Several system parameters influence the "dilution": sample volume,

coil length and diameter, number and nature of merging streams, flow velocity and geometry, etc. General guidelines for system design to control sample dispersion in single-phase systems have been treated in detail in Chapters 2 and 5. Most of these guidelines are valid also for two-phase systems but additional aspects appear. For simple manifolds the "dilution loss factor" might amount to 3 - 4 for a sample volume of 40 μl, i.e., the analytical signal is a factor 3 - 4 lower for the flow process in comparison with the batch process.

What is causing the analyte dispersion in a flow injection extraction system? To answer this question a fundamental study of the segmented stream in the extraction coil is necessary. It has been shown that organic solvents form a film in the PTFE extraction coil and that the properties of this film is vital for the extraction process (4).

The film thickness is typically 0.03 mm for the pentanol/water system at a linear flow velocity of 5 cm/sec. The film thickness increases linearly with the linear flow velocity. The thickness of the film further depends on the nature of the organic solvent. More specifically, the ratio between the viscocity, η , and the interfacial tension, γ , of the solvent can be used as a quantitative estimate when comparing different solvents. A thin film is characterized by a low η/γ ratio. Since the organic phase forms a film on the PTFE tube wall, all aqueous segments will be surrounded by organic phase. The analyte species present in an aqueous segment may then enter either into the film region or directly into the bulk region of the organic phase.

Fig. 6.6 shows the intrasegmental mixing pattern and the film formation in a PTFE tube. The intrasegmental mixing is extremely efficient with respect to time.
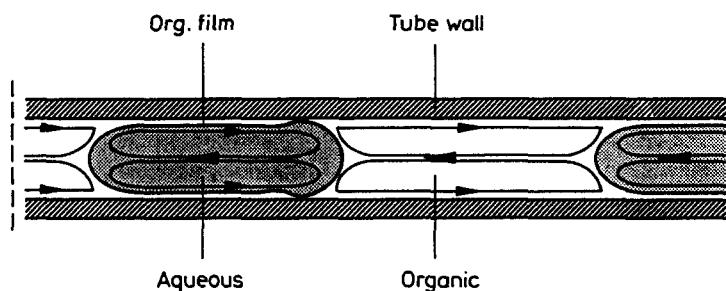
Fig. 6.6. Mixing pattern of a segmented stream in a PTFE tube.

If an analyte species first enters the film region, its linear flow velocity slows down due to the stagnant nature of the film. Eventually, it may migrate into the bulk region of an organic segment passing by the film region. If this latter migration step is delayed so that several segments of organic phase pass by before the analyte species finally leaves

its position at the tube wall, analyte dispersion results. Assuming that extraction takes place from an aqueous phase to an organic phase, the analyte is originally present in a limited number of aqueous segments in the extraction system since a limited amount of sample has been injected. The decrease in linear flow rate caused by the intermediate residence of the analyte in the organic film will then cause increased axial distribution; thus decreasing the analyte concentration in each organic segment leading to a less sensitive method. A thin film causes less axial distribution in comparison with a thick film. This is due to the decreased residence time for the analyte species in the organic film which in turn depends on the shorter migration distances in the thin film.

Fig. 6.7 illustrates the process described above.

FLOW DIRECTION
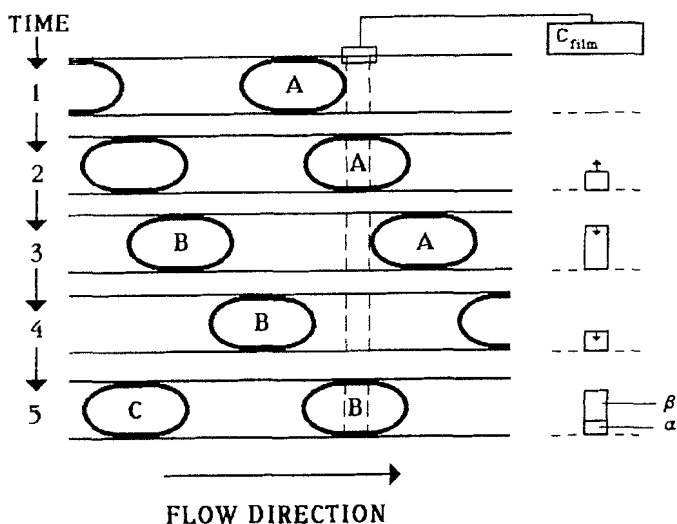
Fig. 6.7. Analyte concentration in the film region, C_{film}, at different times, 1 - 5. A, B and C are aqueous segments passing by the observation area denoted by broken, vertical lines. α is the analyte concentration originating from segment A, β originates from segment B. For details, see text.

At time "1" the aqueous segment A is about to enter the observation region marked by two broken vertical lines. The concentration of analyte, extracted from the aqueous to the organic phase, increases in the organic film region at time "2" up to a maximum (time "3"). When the A segment has left the observation region the analyte in the film can enter the bulk of the organic phase by diffusion and convection processes. As a consequence, the analyte concentration, in the film region drops (time "4") but there may still be excessive analyte material left in the film region when the next aqueous segment (B) approaches (time "5"). Analyte material originating from segment A may be present

not only in the organic phase segments between A and B but also between the B and C segments, the C and D segments, and so forth. Axial sample dispersion results. The axial spreading of sample material will lower the peak height registered by the detector. If the analytical evaluation is based on peak height the result will be interpreted as a loss in sensitivity. The thicker the film the larger the effect of axial dispersion.

What happens if the opposite situation occurs, namely that the analyte originally is present in the film-forming phase? Actually, no further axial distribution of analyte is to be expected after the point where the extraction has reached completion. Before that point, however, analyte dispersion is likely to occur. After that point the segments carrying the analyte are efficiently insulated by the film-forming phase. Transport may be performed over a large distance without changing the analyte concentrations in each individual segment. The phase with the strongest affinity to the tube material forms the film. Glass and steel extraction coils can be used in order to force the aqueous and not the organic phase to form the film.

The reproducible (but variable) physical conditions in the extraction coil in a flow injection extraction system provide a unique means to study the overall extraction rate. If the extraction to be studied is analyte extraction from an aqueous phase into an organic phase, PTFE is used as the extraction coil material. The aqueous segments then are surrounded by the film-forming organic phase. A large phase boundary area increases the probability for the analyte to cross. Narrow extraction coils can be employed to increase the phase boundary area. In fact, the overall extraction rate depends mainly on the magnitude of this phase boundary area present per phase volume unit (5).

What are the effects of shorter segment lengths? Shortening the segment length has rather little effect on the magnitude of the phase boundary area. If the segment length is decreased from 38 to 3 mm in a flow injection extraction system, the phase boundary area increases only about 6%. In spite of this moderate increase the extraction rate is enhanced. This enhancement can be explained by improved mass transfer between the bulk and the stationary organic layer due to eddies appearing at points on the tubing wall situated just behind the water segments. When the number of segments is increased the number of such eddy points correspondingly increases. There are indications that the intrasegmental mixing can be enhanced to a level at which the extraction rate will depend on the extraction kinetics only (5).

Different modes of analyte transfer will now be discussed in detail. But first, a fundamental question that has to be asked is: why are liquid-liquid extractions performed at all? Liquid-liquid extractions are performed in order to transplant the analyte into a new matrix. The analyte is transferred from one liquid matrix to a second liquid matrix. The relative volume of the two immiscible phases involved may furthermore favor analyte enrichment. But the matrix shift per se may also result in an enhancement of the selectivity. Undefined matrices which might interfere during the detection step are

exchanged for a clean, uniform and well-defined matrix. The noise level decreases and the signal to noise level increases; the practical limits of detection improve. In some simple applications there are alternative analyte transfer approaches available based on the flow injection principle rather than the original one. The first one, shown in Fig. 6.8 is similar to dialysis or gas diffusion (6).
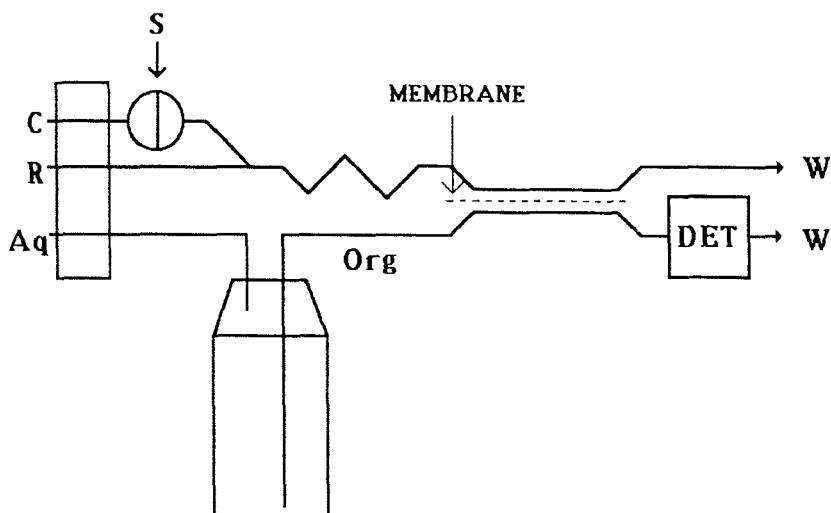
Fig. 6.8. Flow scheme for extractions in a flow system without liquid-liquid segmentation. C = carrier, R = reagent, S = sample, Aq = water supplied to displacement bottle, Org = organic phase, DET = detector, W = waste.

The sample is injected in the aqueous carrier, C, and passed through an extraction cell in which a PTFE membrane is placed so that the aqueous stream flows along one of the two sides of the membrane. The organic phase flows by the opposite phase through the membrane. The yield is only 8 - 18% in comparison with liquid-liquid segmented extraction. The approach in Fig. 6.8 can be applied when concentrated samples are to be analyzed. The carrier stream containing the sample can, of course, be mixed with a reagent stream prior to introduction into the extraction cell. An interesting aspect of this technique is that the two liquids separated by the membrane can be partly miscible. For instance, the sample matrix can be comprised of a 40/60 methanol/water mixture and the analyte receiving liquid a 50/50 chloroform/methanol mixture. Analyte transfer occurs without any change in bulk composition of the two liquid mixtures involved. A similar system with stagnant organic phase present in the membrane has been described for the analysis of amines (7).

Another possible way to perform a matrix shift combined with analyte enrichment is illustrated in Fig. 6.9. This approach is very similar to analyte enrichment using ion

exchange packed-bed reactors. But instead of ions neutral molecules are trapped in a packed-bed reactor filled with reversed-phase octadecylsilane (C_{18}) bonded silica gel. By subsequent introduction of methanol, for example, the analyte is released and can be brought to the detector. The liquid-liquid extraction is consequently performed via a solid intermediator. This liquid-solid-liquid extraction technique is still in its infancy and prediction about its practical applicability is delicate. The main merit of this approach is its simplicity which would make it attractive for qualitative and semi-quantitative determinations where the demand for a short response time and a fast answer is stressed.
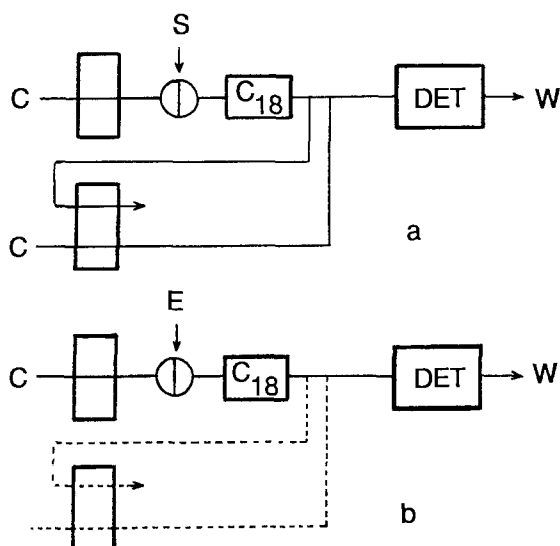
Fig. 6.9. Liquid-solid-liquid extraction. a - trapping of analyte, b - eluation of analyte.
C = carrier, S = sample, C_{18} = C_{18} column, DET = detector, E = eluent, W = waste.

6.3   GAS DIFFUSION

The best transplantation technique for gaseous analytes, or those analytes which can be converted to a gaseous species, is gas diffusion. The gas diffusion technique was extensively used as a batch procedure in the pharmaceutical industry before the advent of chromatography. Under batch conditions, reproducibility of the gas diffusion technique is difficult to maintain. Therefore, the extra time to carry out the chromatography was justified since the reproducibility increases.

There are a large number of analytes that can be measured in a gas phase process at room temperature and atmospheric pressure either directly or after analyte conversion. The major advantage of the gas diffusion process is that it removes the analyte of interest from the sample matrix. The analyte is transferred to a new matrix, the acceptor stream,

which contains no chemical or physical interferents. An additional advantage is that the acceptor stream can be configured so that the optimized conditions for the detection process are realized. For example, the optimized reaction conditions for the formation of the analyte/reagent complex can be maintained in the acceptor stream.

A typical gas diffusion manifold is shown in Fig. 6.10.
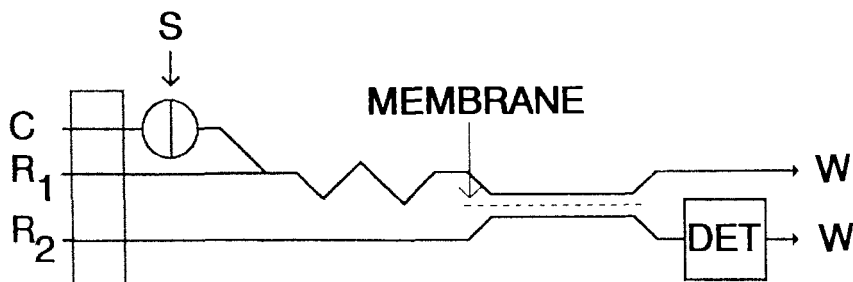
Fig. 6.10. Typical gas diffusion manifold. C = carrier, S = sample, R_1 and R_2 = reagents, DET = detector, W = waste.

The sample is injected into a carrier stream. If any chemistry has to be carried out before the gas diffusion process, the carrier stream is merged with the appropriate reagents. The entire manifold below the membrane of the gas diffusion cell is called the donor stream. When all the necessary pretreatments of the donor stream have been accomplished, the donor stream is passed under the gas diffusion membrane where the gaseous analyte or high vapor pressure compounds can diffuse through the membrane into the acceptor stream. The acceptor stream may or may not contain reagents. This is dependent on the analytical method and detector being utilized. However, it is important to realize that the acceptor stream can be optimized to maximize the observable signal. The acceptor stream may be merged with the appropriate reagents to produce the expected analytical signal. Sample matrices range from water to whole blood.

Several questions concerning the optimization of the gas diffusion manifold have been raised. Van der Linden attempted to explain the observed behavior of gas diffusion by developing a mathematical model (8). This model uses the "Tank in Series" approximation. This treatment assumes that the concentration is uniform across the entire diameter of each segment. The FIA system is treated as if it were at equilibrium. Two extreme cases were tested: a) where both vapor pressure differential and membrane permeability is low, and b) where both vapor pressure differential and membrane permeability are high. In the low cases a direct proportionality between peak height, initial sample concentration, and residence time with an inverse proportionality to flow rate is

predicted. In the high case only peak height is predicted to be related to concentration. Essentially, diffusion of the compound from the bulk solution to the solution/membrane interface, partition between donor solution and membrane phase, diffusion inside the membrane, partition between membrane and acceptor solution, and finally, diffusion from the surface into the bulk of the acceptor stream were grouped into a "constant term". Clearly, several practical factors of the gas diffusion process concerning kinetic rates of diffusion, the non-equilibrium conditions and gas solubility were ignored.

The real problem in modelling an FIA system is that none of the current theories completely predict its behavior. When an additional process, like gas diffusion is added, the modelling becomes difficult at best. However, it is clear that a practical guide to those analysts interested in using gas diffusion flow injection analysis, GD-FIA, would be helpful.

How much gas is currently transported through the membrane? The surprising answer is that 5 - 20 % of the gas is transported. Therefore, it should be possible to make improvements in the efficiency of from 2.5 to 10 times more gas diffusion. How can this be accomplished? To start with, what kind of chemical system should be used to study the effects that parameters have on the efficiency of gas diffusion? Simple dye experiments cannot be used since the dyes do not act as gases. Therefore, a gaseous analyte must be used. However, this analyte must not require any chemistry to be carried out in order to detect it. This is necessary so that only the physical effects of gas diffusion and no chemical effects are observed.

For the following discussion chlorine dioxide was utilized as the gaseous analyte since it fulfills the above mentioned requirements. The chlorine dioxide was monitored at 300 nm using a flow through UV-visible spectrophotometer. The system utilized a Goretex 0.45 μm pore size teflon membrane. There were absolutely no chemical reactions taking place in the system. Using this system to answer the question about gas diffusion parameters, what is the obvious parameter to investigate? Flow rate and the ratio between the flow rates of the donor and acceptor streams is the answer. It has been observed that under stopped-flow conditions the amount of analyte transported through the membrane increases. This is especially true for chemical systems where the gas reacts with the chemical(s) in the acceptor stream and is irreversibly destroyed (e.g. O_3 with indigo blue) or the gas is converted to another species (e.g. $Cl_2 \rightarrow OCl^-$). However, the actual gain in signal for highly reactive gaseous analytes between slow flows, 0.22 ml/min and stopped-flow has been observed to be small. The gain to the analyst is even smaller if time and high throughput is a significant consideration. For example, at 0.22 ml/min the relative amount of chlorine dioxide which passes through the membrane is 3.9 times the chlorine dioxide which passes through the membrane at 1.4 ml/min. Therefore, it appears that slower flow rates are better than faster rates.

But what about varying the flow ratio between donor and acceptor streams? At first glance it would appear that a faster donor stream would enrich the acceptor better

than a slower donor stream, the idea being that more sample would pass under the same segment of the acceptor stream, thereby almost producing a preconcentration step. The reverse case of slow donor stream and fast acceptor stream could be argued in terms of improved diffusion yield. The truth is that neither condition is optimal. The flow rates of the acceptor stream and the donor stream should be equal! For example, when both donor and acceptor streams are equal, 0.79 ml/min, the relative amount of chlorine dioxide transported is 1.50. If the acceptor stream is slowed to 0.22 ml/min, the value is 1.28. At a faster acceptor stream flow rate of 1.4 ml/min the value is 1.00.

At this point most analysts would say that the flow rate questions are finished. But what about concurrent versus countercurrent flows? Table 6.1 shows the data obtained for the flow rates 1.4, 1.2, 0.79, 0.49, and 0.22 ml/min. These flow rates were used both in concurrent and countercurrent flows and in the differential flow studies. All data was normalized to the 1.4 versus 1.4 ml/min concurrent flow data which was designated as 1.00. The data was the absorbance of chlorine dioxide observed for the given flow system.

TABLE 6.1

Relative amount of chlorine dioxide transported as a function of flow rate and flow direction.

	Acceptor	1.4	1.2	0.79	0.49	0.22	ml/min
Donor							
1.4		1.00	1.13	1.20	0.921	0.782	concurrent
		1.11	1.18	1.17	0.908	0.854	countercurrent
1.2		1.14	1.27	1.28	1.19	0.927	concurrent
		1.21	1.25	1.31	1.21	1.06	countercurrent
0.79		0.975	1.44	1.50	1.50	1.28	concurrent
		0.997	1.45	1.59	1.63	1.52	countercurrent
0.49		1.03	1.72	1.98	1.97	1.92	concurrent
		1.11	1.90	2.06	2.15	2.14	countercurrent
0.22		0.513	1.63	2.08	2.88	3.90	concurrent
		0.548	1.74	2.21	3.06	4.17	countercurrent

The countercurrent flow slightly enhances the observed signal. It would appear that the entire enhancement is due to the change in band broadening and not to any increase in the amount of gas transported through the membrane, i.e. membrane transport. The highest signal was obtained for the 0.22 by 0.22 ml/min flow rates. This is not too surprising. By slowing down the flow rates, the amount of diffusion that can occur should be larger. The peak shapes appear to be similar, therefore the flow rate effect is due primarily to the increase in membrane transport created by the slower flow rates which in turn increase residence time. One general observation was that a slow donor flow rate and a faster acceptor flow rate was more favorable than a fast donor stream and slower acceptor stream.

Would differential pressure between the acceptor and donor streams provide any advantage? A pressure was applied on one side of the membrane. The pressure on both sides of the membrane can be measured and the observed absorbance of chlorine dioxide recorded. Fig. 6.11 shows these data.
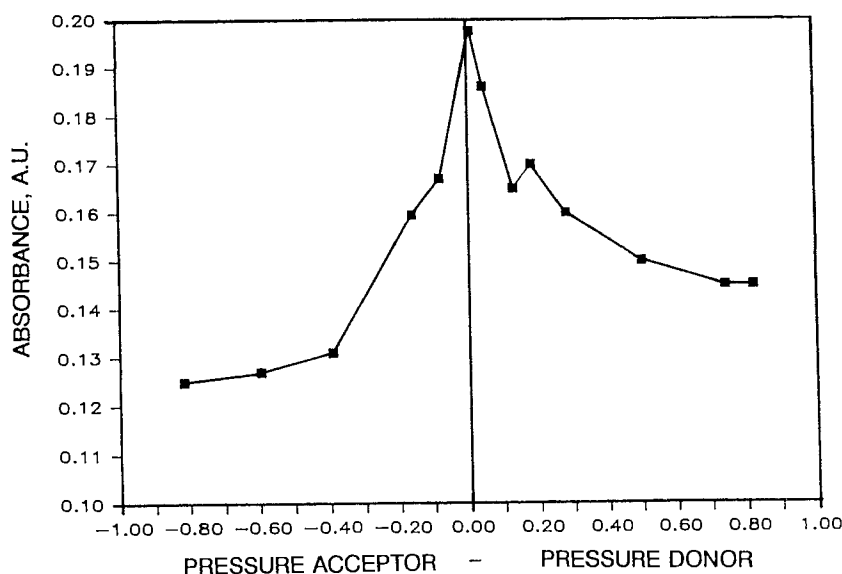
Fig. 6.11. A plot of membrane transport as a function of differential pressure on the acceptor and donor sides of the membrane.

The unsupported membrane clearly exhibits the best transport properties when the pressures on both sides are equal. Using a supported polyethylene scrim backed membrane to eliminate membrane stretching into the channel, similar behavior was

observed. Thus, the pressure should be kept equal on both sides.

Would an increase in membrane area improve the efficiency of the gas diffusion system? Using a simplistic view, it would be expected that the amount of gas transporting through the membrane would exhibit a linear relationship with membrane area. In other words, if 10% of the total gas concentration normally passes through the membrane, then 5% passes through when only 50% of the membrane has come in contact with the sample. Fig. 6.12 shows that for chlorine dioxide, the rate of transport is not linear, but in fact more rapid in the first half of the membrane. A similar plot is observed for ozone and ammonia. However, for chlorine the rate of transport is slow in the first half of the membrane.
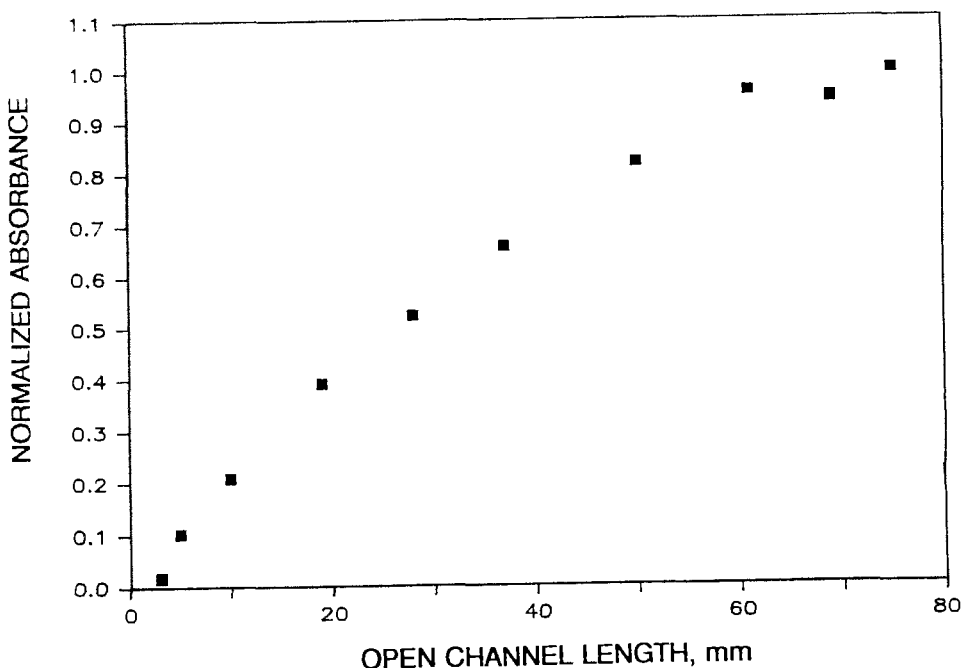
Fig. 6.12. Rate of gas diffusion versus membrane length for chlorine dioxide and chlorine.

This means that a type of kinetic discrimination can be incorporated into an analytical method, and/or manifold design utilizing this transport difference. It should be possible to use this type of phenomenon to develop methods which could directly determine the concentration of both gases without interference from one another.

Given the results on membrane area the next question should be: does channel shape and/or depth play a significant role in the efficiency of the gas diffusion system? Commercial gas diffusion manifolds have a straight channel which is long and deep. Given recent findings (4) concerning the mechanism of FIA extractions, it seems reasonable to hypothesize that similar fundamental properties should affect the efficiency of gas diffusion. For example, the donor stream which is under the membrane can be divided into four separate and operationally different layers. First, the bulk solution itself, second the few molecule thick layer against the membrane, third the gas in the membrane itself and lastly, the few molecule thick layer against the membrane on the donor side. The physical transport of the gas is driven by partial pressure difference between the two sides of the membrane and any differences in the solubility of the gas in the donor and acceptor streams. The actual physical process is molecular diffusion. Therefore, only the second layer, membrane interaction in the third layer, and the fourth layer play a role in the efficiency of the gas diffusion process. The diffusion coefficients for the gaseous analytes are usually 1000 times larger than those for analyte ions or molecules in solution!

Assuming this mechanism is correct, what parameter do we need to maximize? The surface area between sample and membrane should be the answer. How can this be accomplished? The gas diffusion channel can be widened. This would increase surface area, however, the practical aspect of keeping the membrane intact, i.e. not collapsing into one side or the other, cannot be easily resolved. Therefore, the only choice is to lessen the depth of the channel thereby decreasing the ratio between the first and second layer which in effect increases the amount of the second layer. The difficulty with this is that flow rates will increase linearly. This means that there is a trade off point between a reduced channel depth and an increase in linear flow rate.

Comparison of normalized absorbance versus channel depth for the spiral and serpentine channel shapes shows that the spiral shape improves gas diffusion efficiency. It is also obvious that the more shallow the channel the better gas transport in both designs. The nonlinearity is due to the channel depth/flow rate trade off. The spiral and serpentine design both add turbulence to the system. This turbulence mixes the sample plug, thereby replenishing some of the second layer.

At this point all the important parameters effecting the efficiency of gas diffusion FIA have been evaluated. Any parameter which can be optimized to improve transport would improve the sensitivity of the analytical method. Therefore, in conclusion, pressure differential should not exist in order to maximize signal. Countercurrent flow only slightly improves signal (less than 10%) and mostly through peak shape changes and not improvement in gas transport. Flow rates should be equal and as slow as possible. Stopped-flow helps but may not be worth the extra time. The membrane area may be used to differentiate between gases. Finally, channel depths should be as shallow as possible and the spiral shape for a manifold is the best.

Obviously, the limitation of gas diffusion is that the analyte must be capable of being removed from the donor stream as a gas. At first glance this might seem to limit the application of gas diffusion. However, converted analytes or reaction products such as hydrogen peroxide and highly volatile organic compounds can be determined in gas diffusion systems.

The real advantage to gas diffusion is the ability to combine chemistry and/or other selectivity enhancement technique in an effort to increase the selectivity of the analytical method. One such example is the determination of chlorine dioxide in recently disinfected water (9). The potential interferents in the sample are chlorine, chlorite ion, chlorate ion, metal ions and peroxides. The detection method used was chemiluminescence with luminol. The selectivity needed for chlorine dioxide over the interferences is at least 500 to 1. The metal ions and peroxides are eliminated as interferents by the gas diffusion process, since they will not pass through the teflon membrane which will not be wetted by the aqueous solution. Only chlorine and chlorine dioxide will transport across the membrane. However, the chlorine dioxide passes through the membrane at a preferential rate of 3.1 to 1 over chlorine.

The luminol chemistry itself can be manipulated in such a way that identical concentrations of chlorine dioxide and chlorine will produce signals that are 500 times more intense for chlorine dioxide. Since these selectivity enhancement factors are multiplicative, the overall selectivity is greater than 1500 (3.1 x 500) to 1 in favor of chlorine dioxide. Therefore, by incorporating gas diffusion FIA into the chemiluminescence method for chlorine dioxide, a highly selective method with excellent reproducibility is possible.

6.4 DIALYSIS

Dialysis is a selectivity enhancement technique which is useful for the separation of the analyte of interest from the sample matrix. The observed selectivity of this technique is primarily dependent on the properties of the membrane used in the dialysis manifold. The usual system will have a membrane which is capable of transporting ions. In theory, any ion of interest can be separated from the matrix. For example, potassium can be separated from the proteins and many of the large molecules in blood serum. Unfortunately, most membranes which are currently available to analytical chemists are not very selective. Therefore, in the blood serum case, not only potassium ions but other ions, e.g. sodium and calcium ions, will also pass through the membranes. The dialysis process can also be seen as a way to dilute a sample.

Essentially, the same design considerations discussed for gas diffusion hold true for dialysis with the notable exception that the membranes are different. Whereas gas

diffusion uses hydrophobic microporous membranes, dialysis uses hydrophilic membranes like cellulose based membranes. The efficiency of the dialysis system can be improved if a spiral type manifold is used. In addition, the depth of the dialysis channel should be kept as small as possible. In cases where efficiency is a concern and a commercial gas diffusion unit is used, all one has to do to carry out dialysis is fill the channels with small glass beads. This will increase the surface area of the sample membrane interface.

An additional technique employed to apparently increase efficiency, but in fact is a preconcentration step, is to fill the acceptor stream channel with ion exchange resin. For example, by using a cation exchange resin potassium ion and other monovalent ions would be preferentially retained while multivalent cations to a large extent remain in the acceptor stream and pass on to waste. The sample can be readily injected into the stream which continuously passes under the dialysis membrane. Once the desired preconcentration is achieved, the composition of the carrier stream can be changed in order to exchange the analyte of interest of the resin.

Essentially, dialysis is only limited by the types of hydrophilic membranes which are commercially available. The selectivity of these membranes can be modified by coating or immobilizing an active reagent, such as an enzyme or ionophore, on the surface of the membrane. The altered membrane will then exhibit additional selectivity. Just as in any immobilization situation, the lifetime of these devices is always a concern. However, the use of flow injection extends the lifetime of these systems. Another advantage is that reconditioning can be performed continuously by an appropriate choice of carrier solution.

6.5   PACKED-BED REACTORS

The difficulty with discussing packed-bed reactors used in FIA is that they have such a variety of functions. Packed-bed reactors can be used to preconcentrate the analyte before the determination. In some cases the analyte must be converted to another species which is directly determined by the analytical method. The reagent may be immobilized in packed-bed reactors to increase the cost effectiveness or the efficiency of the chemistry. They can be used to modify the sample matrix by trapping the analyte of interest or the interferent, thereby separating the matrix components. They may even be used to generate the reagent. In each case the function of the packed-bed reactors dictates the size, the manifold design, and the lifetime. Consequently, there is continual discussion about the best packed-bed reactor design for FIA.

By placing a packed-bed reactor in an FIA manifold: what concerns should be raised? As always, the first concern is whether the dispersion properties of the FIA will change or not. It should be pointed out that the packed-bed reactors used in FIA are not the size of the packed-bed reactors used in HPLC. By now, it is obvious that an increase

in dispersion means increased dilution of the sample zone. If the detection limits of the method are close to the desired levels, then any additional dispersion is undesirable. However, all too often the discussions about reactors center around mixing, which in turn leads to confusion between dispersion and mixing. The bottom line is this. The packed-bed reactor must create enough mixing to allow the reagents to come in contact with the analyte or the interferent with the reactive site on the column. The dispersion should be kept as low as possible. The amount of reaction which takes place depends on the kinetics of the reaction, the total residence time of the analyte in the reactor, and the concentration of reactive sites in the column. It is possible to create an appropriate residence time without significantly increasing dispersion.

The second concern is for reproducibility. The are two parts to the reproducibility question; can packed-bed reactors be made reproducibly and how quickly does the reactivity of the reactor change? The first question concerning reproducibility can be answered as follows. The art of packing a column is at times difficult and each individual analyst will have different success. However, the different packed-bed reactors can be calibrated in the FIA system. A variation in the performance level between reactors of less than 10% is quite acceptable. The second reproducibility question concerns the loss in activity due to reagent degradation or saturation of the reactive sites. This is a more difficult problem. Ideally, the analyst should find the reactor conditions that will minimize this loss. In practice, especially with enzymes, this condition will not be met. Frequent calibrations will be needed to ensure the most accurate results.

What is the best packing method for these reactors? There are two basic methods. The material is packed into the column in an irregular and nonreproducible manner with no channels in the packed material. The second possibility is that the packing material consists of uniform beads which are placed in a zig-zag pattern. This second reactor is called the string-bead or pearl-string reactor. In this case the packing material has a diameter which is 60 - 80 % of the diameter of the tubing material. The packing is placed in the tubing in a zig-zag pattern. Both packing methods have been shown to minimize dispersion and enhance mixing. Dispersion is decreased because the effective tubing diameter is considerably smaller when the tubing is filled with packing material. Smaller tubing diameters result in lower dispersion. The mixing is increased because a considerable amount of turbulence is generated as the solutions impinge the particles. Furthermore, the radial diffusion distances are decreased in a tubing with a smaller diameter thereby improving radial mixing. As long as channels do not exist in the reactor the reproducibility of the reactor mixing is good. The sample gradient profile can be maintained to a large extent.

What about the geometrical shape of the reactor? Three basic designs exist: straight, coiled and knitted. Although the knitted reactor is more difficult to prepare, the sudden bends in this reactor cause a great deal of turbulence. The effect of this

turbulence is that a greater amount of the sample comes in contact with the reactor contents. The actual chemical efficiency of these reactors appear to be the best of the three designs. Additionally, it would appear that the dispersion is actually smaller in the knitted reactor than in the other reactor designs. Unfortunately, the experiments which have been carried out to investigate the dispersion question have not truly resolved this issue. As pointed out earlier, the choice of design will depend a great deal on the intended function of the reactor. If ion-exchange is the function then the extra trouble of making the string-bead is unnecessary. If a long residence time is required, many people believe that the string-bead reactor is the best choice.

Some examples of reactor appplications will now be presented. In the case of the analyte being converted to a species that will react with the reagent, the commonly cited example is the determination of nitrate ion. A copperized cadmium reactor is used to convert the nitrate to nitrite. The obvious advantage of the column is that it allows the reduction to take place without the cadmium particles entering the manifold. This is important since cadmium particles would clog the manifold. In this case, the reagent is relatively reproducible with respect to time. The reactor can be small enough to be in a microconduit type manifold.

Matrix modification is an important technique of FIA which can be performed with reactors. The sample, which contains analyte and potential interferences, is injected into the system. The reactor is packed with a material containing a functional group which complexes with the analyte or the interferences. Let us assume that the packing material retains the analyte. The sample zone enters the reactor where the first few particles trap the analyte. The interferences and other components of the sample matrix proceed toward the waste outlet. After the sample zone has passed through the reactor, the next step is to eluate the analyte from the reactor. Should this step take place in the same flow direction as the sample zone or should the flow be reversed? The answer is that the flow should be reversed. If the analyte zone was passed through the reactor, the zone would be continuously dispersed. By reversing the flow the analyte zone is prevented from being dispersed into a large zone. The reverse flow approach pushes the analyte out the short end of the reactor. Such a system is shown in Fig. 6.13.

The column can also simply delay the analyte from a difficult matrix. This technique might be employed when the highly colored sample matrix exhibits the same absorption as the analyte/reagent complex.

Preconcentration of the sample is possible using reactors in an FIA manifold. For example, an ion exchanger could be packed into a column and the carrier stream composition optimized to retain the analyte of interest. In most cases, a larger than normal sample injection volume is used to enhance the potential gain in total preconcentration resulting in a dispersion coefficient less than 1. In this case, the analyte may or may not be eluted using flow reversal. This decision is dependent on the portion

of the column used for the preconcentration. If greater than 50% of the available binding sites are used it is better to maintain the same flow direction.

COLUMN

S

C

E

DET → W

→ W

TRAPPING STEP

COLUMN

C

E

DET → W

→ W

ELUATION STEP
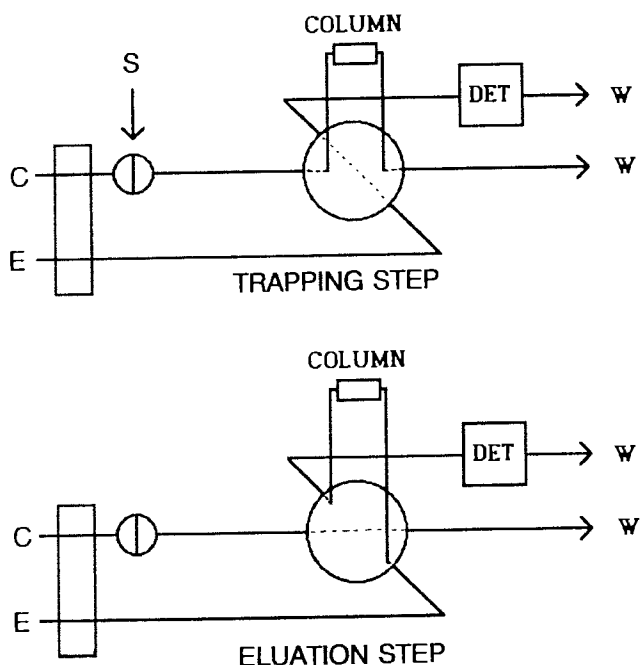
Fig. 6.13. FIA manifold where a packed-bed reactor is used as a matrix modifier and the analyte is eluted by reversing the flow direction. C = carrier, S = sample, E = eluent, DET = detector, W = waste.

The size of the sample used in preconcentration is dependent on the expected concentrations and the meq/l value of the ion exchanger. Care should be taken to ensure that the analyte does not breakthrough the packed-bed reactor. In other words, make sure that the analyte is trapped and that the column has not been saturated.

Immobilized reagents are usually used when the reagent is expensive or when the presence of the first reagent in the manifold will adversely affect the susequent reactions of a method. The classical example is the use of enzymes in an immobilized form. The expense of these reagents makes it imperative that a minimum amount is used. The biggest difficulty in using immobilized enzymes is ensuring that the reactivity is stable. If

the reagent reactivity decreases with time then the necessary and frequent calibration of such a system becomes time consuming. Usually the reactivity can be controlled by the proper choice of carrier stream. It is also possible to eliminate this worry by using easily replaceable reactors. The assumption in this case is that the reproducibility among reactors is good.

Although the few examples exist in the literature for this last potential use of reactors, it is a useful application. The reactor in this case is the generates the reagent. For example, an electrochemically generated reagent which is too unstable in solution to be useful, can be produced inside a reactor. In this case the generation of the material is a function of the applied potential. Then the reagent is free to react with the analyte. Thus far most reactors have generated reagents which are strong oxidizers or reducers. The great advantage is that the reagents need to be stable only for as long as the residence time of that reagent in the FIA manifold. Many of these reagents will be more stable under FIA conditions since the possibility of air oxidation is eliminated. Application of this technique to reagents other than redox systems and to solvents seems to be most promising.

REFERENCES

1.   B. Karlberg and S. Thelander, Anal. Chim. Acta, 98 (1978) 1. [17]

2.   L. Nord and B. Karlberg, Anal. Chim. Acta, 125 (1981) 199. [240]

3.   R.H. Atallah, J. Ruzicka and G.D. Christian, Anal. Chem., 59 (1987) 2909. [1332]

4.   L. Nord and B. Karlberg, Anal. Chim. Acta, 164 (1984) 233. [504]

5.   L. Nord, K. Backstrom, L.-G. Danielsson, F. Ingman and B. Karlberg, 194 (1987) 221. [1408]

6.   Y. Sahlestrom and B. Karlberg, Anal. Chim. Acta, 179 (1986) 315. [742]

7.   G. Audunsson, Anal. Chem., 58 (1986) 2714. [771]

8.   W.E. Van der Linden, Anal. Chim. Acta, 151 (1983) 359. [331]

9.   D.A. Hollowell, J.R. Gord, G. Gordon and G.E. Pacey, Anal. Chem., 58 (1986) 1524. [767]

CHAPTER 7

UTILIZATION OF THE CONCENTRATION GRADIENT

7.1   INTRODUCTION

In Chapter 2.9 the concept of the concentration gradient was presented. The concentration gradient is a unique feature of FIA, generated by the reproducible timing and controllable sample dispersion. The reproducibility of individual points along the gradient is just as precise as that of the peak height. This unique FIA characteristic is the most promising, yet most difficult feature to deal with in terms of understanding the mechanisms and utilizing the potential advantages.

The techniques to be discussed in this chapter include titration, gradient dilution, gradient calibration, stopped-flow, and penetrating zones. In each case, the concentration gradient is created and then exploited to measure some parameter. The degree of dispersion which is allowed to occur is dependent on the gradient technique employed. For example, FIA titrations require a large degree of dispersion while penetrating zones require medium dispersion.

Although some would argue that the gradient techniques are misnamed, the bottom line is that they all work. Some of these techniques work under a very select set of conditions and have not found a practical utilization beyond the research environment. Therefore, one should carefully examine the chemical system for application to the gradient technique.

7.2   FIA TITRATIONS

The oldest application of an FIA gradient technique is FIA titration. For comparison, let us examine a classical wet chemistry titration experiment. A known volume of the sample, e.g. acid, is titrated by a measured volume of titrant with a known concentration, e.g. base. The end-point, which is hopefully near the equivalence point of the titration, is measured as, e.g., a change in indicator color. The titrant volume, V_t, is read. The titrant concentration, C_t, is known. The sample volume, V_s, is known. The sample concentration, C_s, is then easily derived:

$$C_s = V_t * C_t / V_s \qquad (7.1)$$

Can this same experiment be carried out in an FIA system? In terms of procedural similarities, there are points on the FIA peak which are representative of the same equilibrium conditions as the classical titration. At the equivalence point of a titration, the amount of titrant and analyte initially present is equal. Beyond this point the titrant is in excess while before the equivalence point there is an amount of untitrated analyte. The indicator is chosen so that the color change occurs at a point as close to the equivalence point as possible.

A typical FIA titration manifold is shown in Fig. 7.1. It can be used for the determination of acid concentration using base as the titrant.

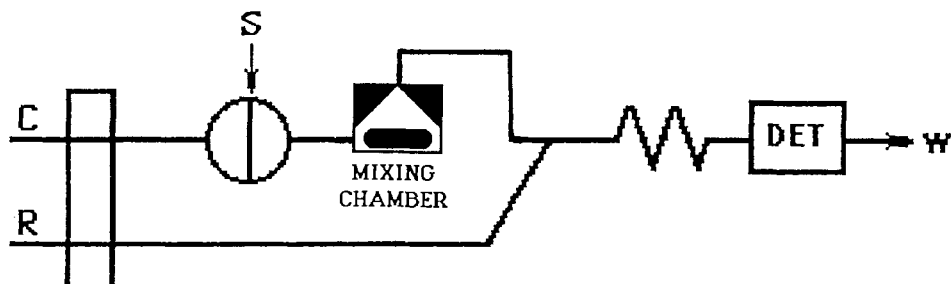Fig. 7.1. Typical FIA titration manifold for the determination of acid concentration. DET is a spectrophotometer. C = carrier, S = sample (acid), R = titrant (base), W = waste.

What would the output of the detector look like for increasing concentrations of acid in the sample? Fig. 7.2 shows the expected result.
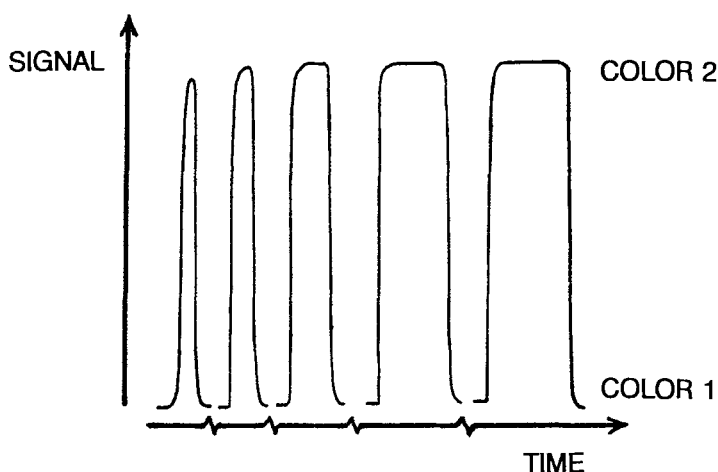
Fig. 7.2. Change in peak shape produced by an FIA titration system with increasing acid sample concentration.

Two important peak characteristics need to be mentioned in relation to Fig. 7.2. The first is that increasing acid sample concentration increases the width of the peak. This width can therefore be used to evaluate the original acid concentration in the sample. Second, the peaks observed in this type of system reach a steady state level area at which all indicating reagent is converted.

The peaks shown in Fig. 7.2 do not represent the true concentration gradient of the sample. In actuality, the typical sample concentration gradient still exists, see Fig. 7.3. The cut-off of the normal FIA peak is due to the indicator reaching maximum color change before the peak reaches its true maximum. Therefore, the resulting change in concentration of the base is not detected. After the peak maximum, the point at which the indicator starts to change back to the original color is the beginning of the observed portion of the peak descent. In essence, the indicator is acting as a yes/no system, see Fig. 7.3. When a detection principle such as potentiometry is used the actual changes in concentration is measured resulting in "normal" FIA peaks, i.e., peaks like those shown in the upper panel of Fig. 7.3.

$$c_{ACID} > c_{BASE}$$
$$c_{ACID} < c_{BASE}$$

ACID IN EXCESS?

— YES — — COLOR 2

NO — COLOR 1
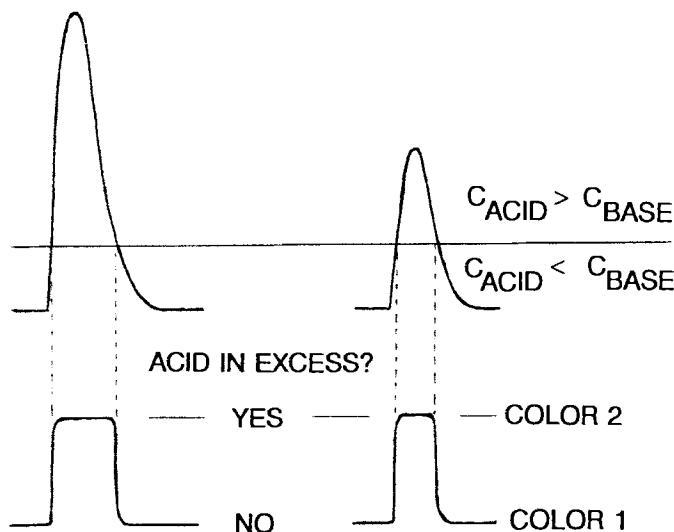
Fig. 7.3. Yes/no titration peaks.

How can peak width be used to evaluate the acid sample concentration? To answer this question, it is necessary to ask more fundamental questions about this technique. To start with, what relationship exists between the time elapsed between any two points of equal color and the original concentration of the injected sample? An estimate of this relationship can be made by a simplified derivation using the acid sample, HCl, and base titrant, NaOH, example. The following terms are defined:

C_A concentration of HCl (sample) injected into the carrier, M

C_B concentration of NaOH, M

Q_A flow rate of HCl, ml/sec

Q_B flow rate of NaOH, ml/sec

V_s volume of injected sample, ml

V_m volume of gradient chamber, ml, $V_s << V_m$

t_{eq} time during which the color of the acid form of the indicator exists

T near residence time of a species in the gradient chamber, sec; $T = V_m / Q_A$

C_A concentration of HCl leaving the gradient chamber at time t, M

C_{A0} maximum concentration of HCl attained in the gradient chamber, M.

The following situation occurs at t = 0 when all the sample has entered the chamber and nothing has left it. If the sample is ideally mixed in the chamber, then:

$$C_{A0} = V_s * C_A / V_m \qquad (7.2)$$

The following further assumptions are then made:

1.   The analyte and the titrant react stoichiometrically.
2.   The solutions in the gradient chamber are well-mixed so that a simple tank-in-series model can be applied.
3.   The mixing pattern in the gradient chamber is reproducible so that a reproducible concentration gradient of the sample is created.

With all these assumptions made the following relationship is obtained:

$$C_A / C_{A0} = e^{-t/T} \qquad (7.3)$$

At t = 0 C_A equals C_{A0} the maximum concentration of HCl attained in the gradient chamber. At the point when the indicator returns to its original color the acid concentration and the base concentration match each other. Thus, the color change occurs at a specific value of C_A, C_{A0}, regardless of the original concentration of C_A. The value of C_{Aeq} depends, for a given manifold design, on the concentration of the base, C_B, only.

By inserting t_{eq} and C_{Aeq} in Eqn. 7.3, the following expression is obtained:

$$C_{Aeq}/C_{A0} = e^{-t_{eq}/T} \tag{7.4}$$

Now the relationship between t_{eq} and C_{A0} can be derived

$$lnC_{Aeq} - lnC_{A0} = e^{-t_{eq}/T} \tag{7.5}$$

$$t_{eq} = T*lnC_{A0} - T*lnC_{Aeq} \tag{7.6}$$

It can be concluded that t_{eq} is related to the logarithm of the sample concentration.

The limiting factor in the use of the titration method is that the theory used to generate the previous equations assumes the ideal tank mixing process. In reality this is never the case. This explains the fact that the calibration curves have the shape shown in Fig. 7.4. The useful range is in the linear region of the curve.
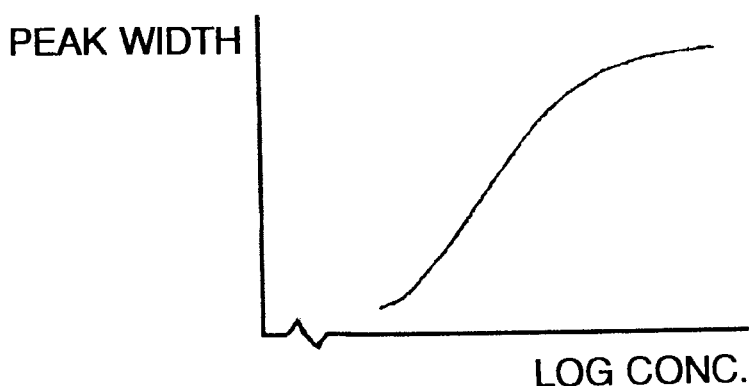
Fig. 7.4. Typical FIA titration calibration curve.

As can be seen in Fig. 7.4 the plot for an FIA titration is peak width as a function of log concentration. The peak width is measured as time or length. The r.s.d. varies as a function of concentration with higher concentrations exhibiting the higher r.s.d. values.

FIA titrations still follow the basic principles of classical titrimetric chemistry. Therefore, polyprotic acids, like carbonic and phosphoric acids or even acid mixtures,

can be titrated under FIA conditions. Varying concentrations of the polyprotic acid will still produce different peak width values. FIA titrations are not limited to acid-base chemistry. Redox, compleximetric and precipitation titrations can also be carried out under FIA conditions. There has been some discussion as to whether FIA titrations are dilutions instead of titrations. The current application of this technique clearly indicates that titrations have occurred in two segments, one before and one after peak maximum. However, it should be noted that there is a practical limitation on the effective range of concentrations over which the FIA titration can be used. Some shifting of the range can be accomplished by varying the titrant concentration.

7.3   GRADIENT DILUTION

In the batch mode, if a sample is too concentrated, it is diluted and the determination rerun. Is it possible to avoid this time consuming and error producing step using the FIA gradient? The answer is yes. Assuming that enough reagent is present to react with only a certain percentage of the sample, the FIA peak would look like the one shown in Fig. 7.5.
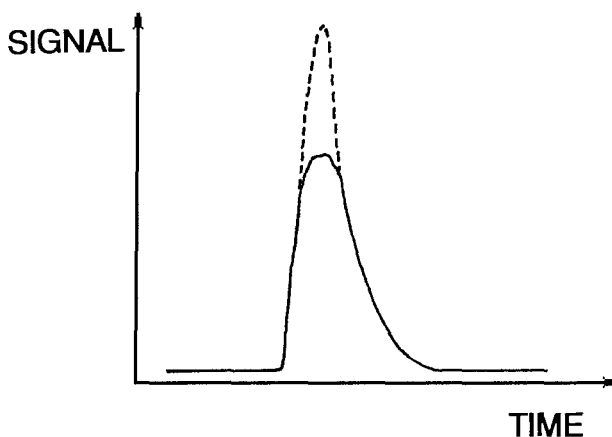
Fig. 7.5. An FIA peak which is caused by nonlinear chemistry/detection or lack of reagent. Solid line is the observed peak, while the dotted line is the expected peak.

As long as the chemistry and the detector system behave ideally in the concentration regions being applied, they will not adversely effect the observed linearity. The potential problems are insufficient reagent(s), inhibition of reactions and nonideal detector response. If there is insufficient reagent to ensure complete reaction or maintain

the kinetic rates, then the sample response curves will be non-linear. Other reaction products could inhibit the formation of the observed reaction product. If any of the points on the gradient are at a level where the validity of Beer's law is in question, then the detector becomes the limiting factor.

As a rule, most of the limitations will occur with high sample concentrations. Therefore, the linearity of response will be affected first at the peak maximum. The tailing portion of the peak will most likely exhibit a linear response. This means that an oversized injection or a highly concentrated sample, which would be off scale at peak maximum, could be evaluated at some other point on the peak.

The technique called gradient dilution was first described and used in 1982 to overcome these problems (1). It is based on the utilization of points along the concentration profile other than the peak maximum. Any of these points can be used to give information as useful as the peak maximum. Therefore, as long as the observation of the signal is made at the same time after the injection, see Fig. 7.6, reproducibility is ensured.
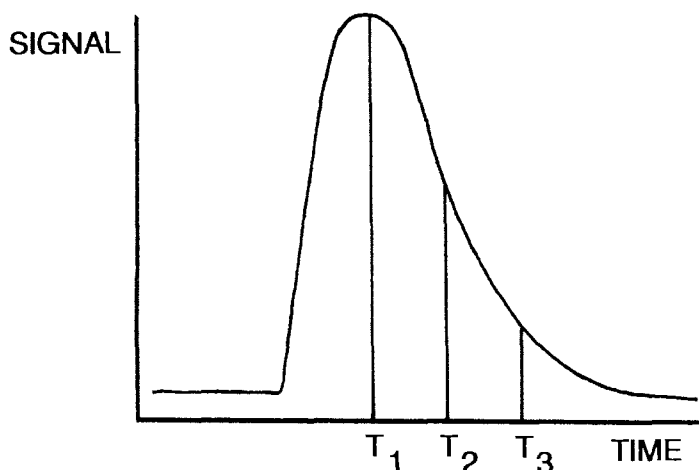
Fig. 7.6. The dispersed sample zone where points T_1, T_2, and T_3 are observation times.

Each T corresponds to a specific dispersion coefficient for the dispersed sample. Consequently, it is possible to produce a series of calibration curves using different points on the FIA peak. Fig. 7.7 shows an example of this principle.
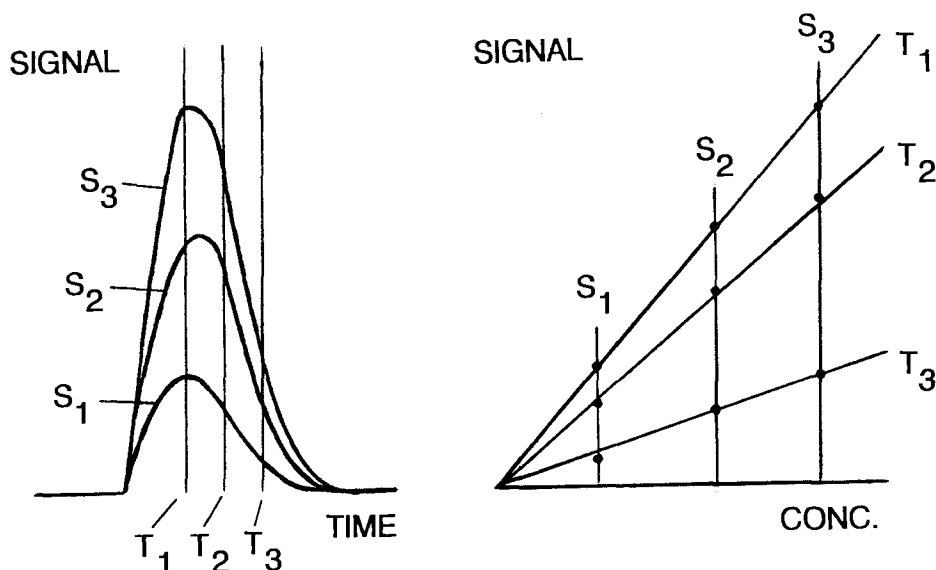
Fig. 7.7. Multiple calibration curves from the same set of FIA peak. S_1, S_2 and S_3 are response curves resulting when injecting corresponding standards. The signal evaluation was made at times T_1, T_2 and T_3.

Utilizing the gradient to produce multiple calibration curves is a logical step since all the information needed is already contained within the FIA peaks. Since each segment contains different relative concentrations of both reagent(s) and sample the net effect is, in fact, a type of serial dilution.

7.4 STOPPED-FLOW

Stopped-flow FIA measurement have been previously discussed in Chapter 5. This technique is used to meet two different objectives. First, an increase in signal can be observed because the residence time is increased. Obviously, the assumption is that the analytical reaction is continuing during the stopped-flow period. Therefore, increased residence time equals increased reaction time, yielding a larger signal until, ultimately, steady state is reached. The sample can either partially reside in the flow cell of the detector or completely outside of the detector cell.

The second objective is to compensate for variations in background signal produced by the potential interferences or differences in the sample matrix. This is best illustrated

by taking an application from the wine industry, the determination of sulfur dioxide by pararosaniline in red wine (2). The sulfur dioxide/pararosaniline complex is red. The sample matrix is also red. The only way to "see" the desired chemistry is to observe the growth of the red signal. The increase is due only to the analytical reaction and not to the background matrix.

Some researchers will argue that the stopped-flow technique also has a third purpose, measurement of reaction rates. This technique may be adequate for first and psuedo first order reactions. However, serious questions remain about higher order reaction rates. Several of the procedural difficulties observed with traditional stopped-flow kinetic experiments may well exist in the FIA procedure. Future research will hopefully answer these questions.

Stopped-flow can be accomplished without a detrimental effect on sample zone dispersion. One of the basic rules of FIA states that in order to increase the residence time the reaction coil should be shortened and the flow rate decreased. Stopped-flow is the ultimate design with no effective coil length and zero flow rate. The only mixing that occurs is the contribution from molecular diffusion. The impact on radial dispersion is significant while it is negligible on axial dispersion. Fortunately, it is the radial dispersion which is desirable in terms of mixing during the stopped-flow time period. This is why a gain in reaction time (and also residence time) is accompanied by no observable increase in dispersion coefficient.

When the sample zone is stopped inside the flow cell, it is possible to record the actual growth of the signal. To utilize this technique in practice, it is necessary to reproducibly control the movement of the carrier stream. Also, the time of the stopped period must be accurately controlled. Reproducible timing and controllable sample dispersion both contribute to FIA's applicability in a stopped flow mode. However, it should be pointed out that the pumps are the critical component in this process. They must be reproducibly stopped at the designated time. Some current pumps do not have this capability.

The sample can also be stopped before the flow cell. This may be necessary when the chemical reaction is affected by the photochemistry which may occur in the cell compartment. It is also advisable to ensure that the change in observed signal is not a result of interaction between reagent(s) and the carrier. Such an interaction would exhibit a baseline change also when the sample is not present in the system.

Fig. 7.8 shows a set of different stopped flow FIA peaks. If no reaction is occurring the observed signal is essentially a flat line parallel to the existing baseline (Fig. 7.8b). However, if the reaction producing the detected species is proceeding, the observed signal increases (Fig. 7.8a). A third possibility exists, namely that the detected species is degraded with time (Fig. 7.8c).

go stop go   go stop go   go stop go

a   b   c
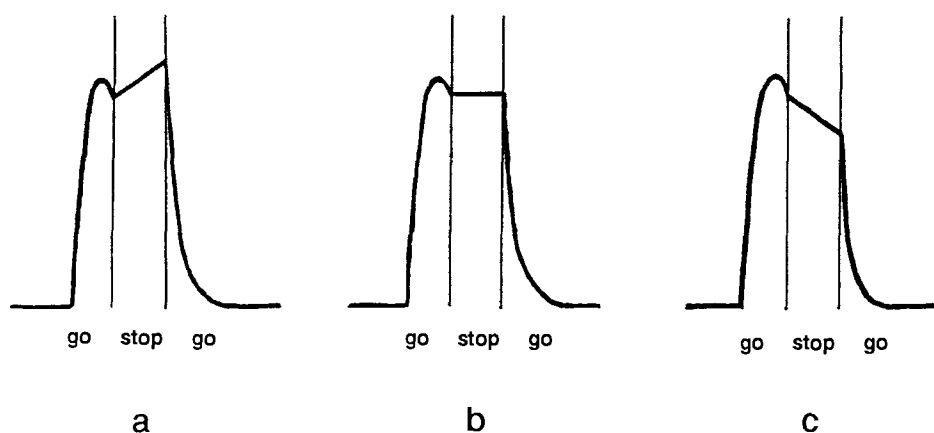
Fig. 7.8. Example of peaks produced during a stopped-flow period. For details, see text.

How is the evaluation of, for instance, the increasing signal in Fig. 7.8a performed? Fig. 7.9 assumes that the increase in signal obtained during the stop period of the pump(s) is due to only the analytical chemistry and not other factors. Therefore, this increase can be related to the analyte concentration. In practice, the relationship between analyte concentration and signal change is not necessarily linear.
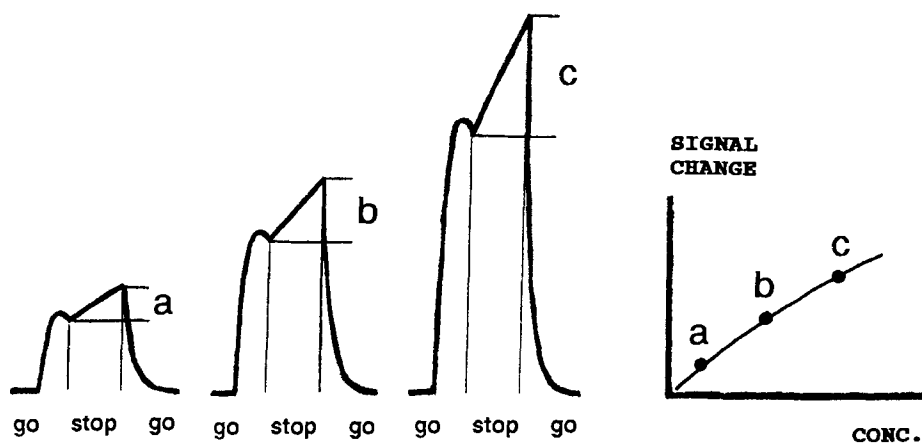
Fig. 7.9. Stopped-flow signal where the analyte continues to react with the reagent. a, b and c designate the signals used for construction of the calibration graph.

7.5   GRADIENT TECHNIQUES BASED ON PENETRATING ZONES (CHASING ZONES)

The most complex concept in the family of gradient techniques is penetrating or chasing zones. Penetrating zones is based upon the intermixing of the gradients formed when two (or multiple) zones are injected simultaneously at different sites in a common carrier. Fig. 7.10a shows the two zones A and B injected as distinct plugs. As the plugs flow downstream the dispersion process results in zone spreading, Fig. 7.10b. Eventually, zone A disperses into zone B, Fig. 7.10c. The overlapped region AB is comprised of the back edge of zone A and the front edge of zone B.
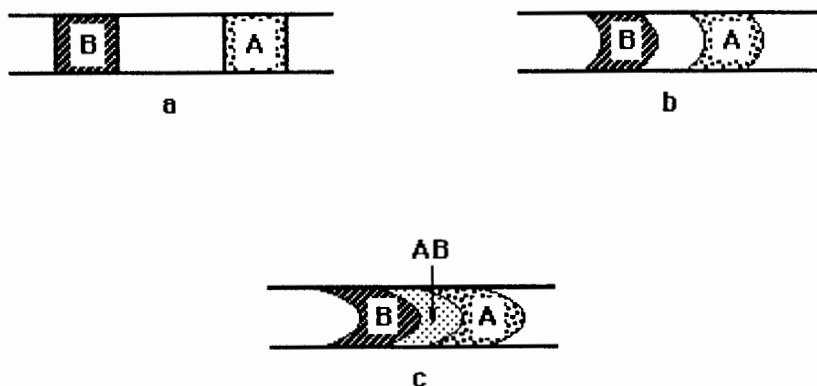
Fig. 7.10. Depiction of the two zones A and B in penetrating zones a) just transferred to the carrier stream, b) initial dispersion, and c) partial overlap (AB).

There are at least two ways to accomplish the insertion of the two zones into the common carrier stream. The simplest way is to use a two-channel injector and a manifold as shown in Fig. 7.11.
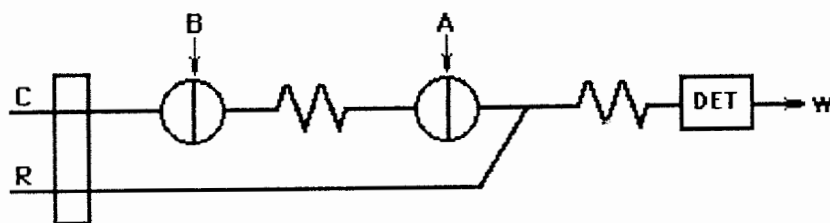
Fig. 7.11. A simple manifold for performing penetrating zones. C = carrier, R = reagent, DET = detector, W = waste. A and B are the injected solutions.

The second approach is to use a one-channel valve with by-pass coils for both carrier and sample, see Fig. 7.12.
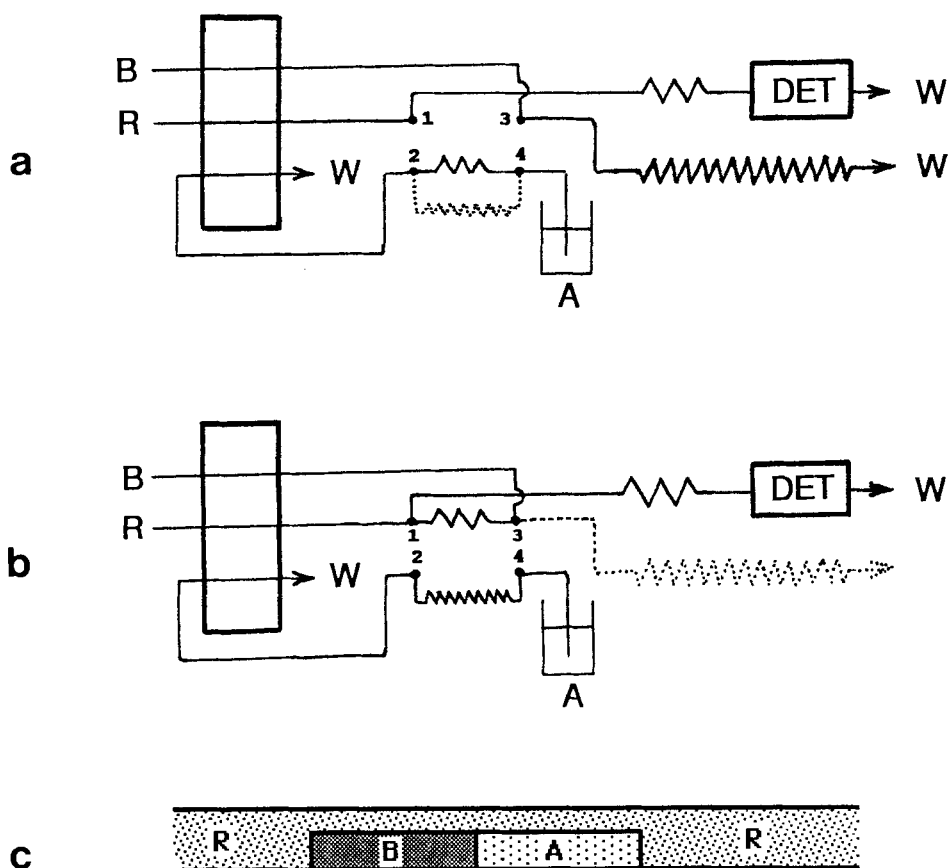
Fig. 7.12. Manifold for performance of penetrating zones using a one-channel valve furnished with by-passes for carrier and sample. For details, see text.

The original by-pass coil for the carrier is removed and replaced with a tube connecting port 1 with the pump tube for R and a restriction coil leading to waste (port 3). Solution B is introduced via port 3. The by-pass coil for the sample is denoted by the broken, horizontal line just above the vessel A in Fig. 7.12a.

In the fill position the solution A is aspirated into the injector loop which connects ports 2 and 4 in Fig. 7.12a. The reagent, R, is used as a carrier and flows directly to the manifold via port 1. Solution B flows via port 3 and the narrow restrictor to waste (or is recollected).

After injection, see Fig. 7.12b, solution B pushes solution A out of the loop, into the reagent stream. The total flow rate through the manifold increases during the time the injector resides in inject position. There must be no flow out of port 3 via the narrow restrictor to waste. The restrictor is dimensioned so that this is fulfilled. Solution A can still be aspirated to waste via the by-pass coil.

Eventually, all solution in the injection loop connecting ports 1 and 3 merges with reagent. At this point, solution B starts to enter the reagent stream and continues to do so as long as the injector remains in inject position. When a sufficient amount of solution B has been introduced, the injector returns to the fill position. The resulting stream is illustrated in Fig. 7.12c. The size of plug A is determined by the size of the injector sample loop and the size of plug B by the time during which the injector is in inject position.

One obvious application of the pentrating zones technique is for selectivity studies (3). Selectivity of any given chemical method is the most important and all too often overlooked aspect of analytical chemistry. Part of this problem exists since the work required to completely understand the interfering chemistry can be extremely time consuming and tedious. Furthermore, in order to determine the extent of interference, a precise and accurate evaluation is required. FIA with its high reproducibility is a natural candidate to improve the way in which analytical chemists might evaluate interferences.

In a pentrating zone experiment the dispersion coefficient, D, will have a definite value at any specific time, t, for each sample or reagent zone. The ratio of D values for the injected zones will remain constant for any value of t as long as the manifold and flow parameters remain unchanged.

Assume that A is the analyte to be studied with respect to the interfering species B. The concentrations of A and B are denoted C_A and C_B, respectively. The measured, apparent concentration of A, C_{APP}, can either be positively or negatively influenced by the presence of species B in the sample. C_{APP} can be related to C_A and C_B by defining a selectivity coefficient, K_{AB}:

$$C_{APP} = C_A + K_{AB} * C_B \qquad (7.7)$$

K_{AB} should be regarded as a conditional selectivity coefficient since its value may be influenced by variations in the sample matrix. It may also change value when C_A and C_B are varied over large concentration ranges.

When A and B are injected in an FIA system, like the one illustrated in Fig. 7.11, any of the measured concentrations C_A and C_B can be related to their original concentrations according to

$$D_A = C_{0A} / C_A \quad \text{and} \quad D_B = C_{0B} / C_B \qquad (7.8)$$

where D_A and D_B are the dispersion coefficients. By injecting pure solution A using either of the two injectors and plotting the two response curves in the same diagram, a point M can be found where the two curves intersect, see Fig. 7.13.
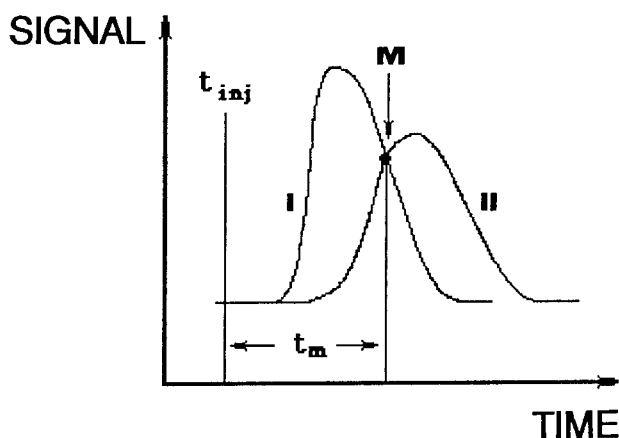
Fig. 7.13. Two response curves, I and II, obtained by injecting pure analyte using one of the two injectors at a time. t_{inj} denotes time of injection, t_m is the time elapsed from injection to attainment of the point M, the intersection of the two response curves.

At this point, the dispersion coefficients for the two curves are equal. Point M can be exactly defined through the value of t_m, see Fig. 7.13.

How is it possible to use the response curve value at time t_m to estimate K_{AB}, conditional selectivity coefficient? The actual experiments are the following. A is injected by injector I. No injection is made by injector II. The response curve value at t_m is H_1. H_1 is directly proportional to the actual concentration of A at this point, C_A, which in turn can be related to the original concentration, C_{0A} :

$$H_1 = K * C_A = K * C_{0A} / D_A \qquad (7.9)$$

where K is a constant.

Next, A is injected by injector I and the interfering species B by injector II. The response curve reading at t_m is H_2 . The following equation is then derived for H_2 :

$$H_2 = K*(C_A + K_{AB}*C_B) = K*(C_{0A}/D_A + K_{AB}*C_{0B}/D_B) \qquad (7.10)$$

If $H_2 > H_1$ the value of K_{AB} is positive, i.e., presence of B in the sample gives rise to too high values of A. It is advisable to evaluate K_{AB} for a complete set of B solutions covering the possible concentration range of this interferent.

The second potential use of penetrating zones is a logical extension of the interference type study. If the second injected zone is a standard instead of an interferent, is it possible to use the observed combined signal as a standard addition type experiment? The answer is yes. If species A is an unknown sample then a known concentration of analyte can be injected as B. The readout is observed at either the point of equal dispersion, where the previous equation is valid, or at any other point along the interface of the two dispersed zone gradients, assuming that the ratio of dispersion coefficients of each selected delay time is constant.

In some cases the penetrating standard zone, B, can be an "infinitely" longer zone than the sample zone, A. Therefore, as long as the sample zone is large enough or the overall dispersion small, the "A" zone is not completely overlapped by the standard zone. The gradient section or the rising part of the sample peak is diluted exclusively by the carrier stream which is free of standard solution. The tailing section is diluted entirely with standard. If the readouts are made at times (points) corresponding to sections having identical dispersion coefficients at the front and tailing parts of the sample zone, t_1 and t_2, the amount of standard added to the sample at t_2 can be determined.

The third potential application for penetrating zones is for identification of mixtures. When using specific detectors such as infrared, many compounds will have absorption peaks at or near the same wavelength. Therefore, some type of preparation step, a hybrid technique like liquid chromatography-IR, can be carried out preceding detection. By combining the techniques of FIA penetrating zones and chemometrics it would be possible to elucidate the components of a mixture by observing the forward state underivatized components and derivitized components in the overlap peak area. This type of approach is not ready for unknown mixtures, but can be routinely used for samples or processes where the composition is known.

REFERENCES

1. S. Olsen, J. Ruzicka and E.H. Hansen, Anal. Chim. Acta, 136 (1982) 101. [263]

2. J. Ruzicka and E.H. Hansen, Anal. Chim. Acta, 114 (1980) 19. [71]

3. E.H. Hansen, J. Ruzicka, F.Z. Krug and E.A.G. Zagatto, Anal. Chim. Acta, 148 (1983) 111. [390]

CHAPTER 8

DEVELOPING AN FIA METHOD

8.1 INTRODUCTION

Is there a trick to converting an existing batch or continuous-flow method to an FIA method? Actually, there is no universal way to develop an FIA method. In many situations the chemical principle applied during the batch method for the samples might still be applicable for the FIA procedure. The actual sample and reagent concentrations can easily be calculated for the batch method mixture. Corresponding concentrations, as well as dilution factors, can be reproduced in the FIA system. All these parameters can be quickly changed in the FIA system to exactly match the batch conditions and/or to optimize the FIA method. In other words, the sample dispersion (or dilution) is controllable. This intrinsic property of FIA was treated at some length in Chapter 2.6.

Flow schemes for air-segmented analyzers are useful when developing an FIA method. The flow ratios, the reaction times and the reagent concentrations provide important information about the automated form of the method. There is a good chance that the conditions for the sample in an air-segmented system can be created in the corresponding FIA system. However, there are methods which are not easily converted. For example, if the air-segmented method requires that the sample is mixed with strong acid and kept in a thermostatted coil at 90 °C for 15 min, it can immediately be concluded that the method is not suitable for FIA. But less complicated methods involving up to three reagents, dilution, dialysis or extraction and with residence times of less than 5 minutes would have a good probability of being converted.

Method development with FIA is usually fast. The response time is short, in most cases 30 - 60 seconds. This means that the consequence of a change in the manifold design can be registered promptly. But there are a lot of parameters that can be changed and they are often interrelated. So, at least in theory, a huge optimization problem is to be solved each time a new FIA method must be developed. Consequently, multivariate methods have been applied (1). In practice, a limited number of experiments is carried out based on experience and intuition rather than on any multivariate model. Often this leads to a method that solves the analytical problem but if the method is to operate at its performance limits then a more careful and scientific approach is recommended.

8.2   CHECK LIST FOR METHOD DEVELOPMENT

The variations in detector parameters can effect the way the FIA method is developed. The following check list, originally described by Moller (2) can be applied to development of a photometric FIA method. The list may be useful for other detectors as well. However, some additional consideration related specifically to optimized detection may be added to this basic list.

Step 1.   Define the analyte. Define the sample matrix. Define the analytical range.

Step 2.   a) Study the literature about the different analyte methods:
- FIA methods (see bibliography)
- air-segmented flow methods
- official methods
- other methods
b) Study the literature about method performance and properties:
- chemical principle
- selectivity
- sensitivity
- analytical range
- interferences
c) Study the literature about sample pretreatment procedures and requirements.

Step 3.   Judge the following aspects for the method choice:
- working range/sensitivity
- selectivity
- sample matrix variations
- interferences
- reaction rates
- approval by regulatory agencies
- possibility to fit the method to FIA
- availability/price/toxicity of reagents
- availability/price/flow through possibility of the detector

Then make the method choice.

Step 4.   Design an FIA manifold for the chosen method and for the choice of reagent
           concentrations. Aspects like dispersion (dilution), working range, sensitivity,
           sample throughput, expected matrix effects, etc. will determine the design.
           Some guidelines:
           - for maximum sensitivity, increase the flow rate of the carrier and decrease
             the flow rates of the reagents, and increase the sample volume (up to
             200 μl).
           - to minimize the influence from the sample matrix, match the composition
             of the carrier to that of the sample (pH, ion strength, etc.)
           - to maximize sample throughput, decrease the sample volume, increase the
             total flow rate, decrease the coil length and coil diameter.

Step 5. Perform the first tests of the designed manifold:
           a) baseline stability - are the carrier and reagents efficiently
           mixed?
           b) determine the dispersion coefficient of the system using a dye (see
           Chapter 2.6).
           c) inject a standard - is the observed signal reasonably large?
           Compare with the batch method.
           d) measure the pH in the waste line - is it within the expected limits?
           e) scan an absorbance spectrum of the reaction product with blank
           correction - is the selected wavelength appropriate?
           f) check the peak shape - is the mixing complete? Is the carrier adapted to
           the standard/sample?
           g) perform a stopped-flow experiment - does the absorbance value increase
           during the stop period? Has the reaction reached completion?

Step 6.   Check the influence of pH on the reaction. Observe the signal response at
           different pH-values. Stopped-flow measurements should be performed and
           the absorbance characteristics registered.

Step 7.   Check the influence of temperature on the reaction, for example at 25, 35 and
           50 °C.

Step 8.   Optimize the manifold design with respect to working range, linearity, sample
           throughput, reproducibility, etc. using the following variables:
           - injection volume
           - carrier flow rate
           - carrier composition

- reagent flow rate
- reagent composition
- coil length
- coil diameter
- instrumental settings
- temperature

Follow up changes in manifold design by stopped-flow and dispersion measurements.

Step 9.   Check the influence of interfering species. Spike a standard (or sample) solution with the interferent to be investigated.

Step 10.   Check the validity of the method:
- analysis of actual samples
- recovery studies
- reproducibility studies
- stability tests (both samples and reagents)
- analysis of standard reference material
- comparison with reference methods
- interlaboratory tests.

8.3   HOW TO USE THE CHECK LIST FOR METHOD DEVELOPMENT - AN EXAMPLE

The check list in the previous section is now going to be used to develop an FIA method for the determination of iron in water.

Step 1.   The analyte is iron. The sample matrix is well, spring, surface or drinking water. The concentration range is 0 - 1.0 mg/l.

Step 2.   Both continuous flow and FIA methods for iron have been described in the literature. Colorimetric methods using o-phenanthroline (A) and TPTZ (2,4,6-Tri-2-pyridil-1,3,5 triazine) (B) are standard methods in several countries. Ferrozine (3-(2-pyridil)-5,6-bis (4-phenylsulfonic acid)-1,2,4 triazine, disodium salt) is a relatively new reagent (C). A comparison of the molar absorptivities shows that an FIA method based on Ferrozine should result in a more sensitive method.

Reagent	Molar absorptivity
A	11,100
B	22,600
C	27,900

The iron-Ferrozine complex should also have a good water solubility. The ferrous ion, Fe^{2+}, requires six electrons to complete its 3d orbitals and possess a stable electron configuration. Therefore, it is likely that three Ferrozine molecules complex the ferrous ion octahedrally in a cis-configuration.

At low pH-values, the nitrogen sites are deactivated. Buffering to a pH value of about 4.5 is necessary (acetate buffer). Other metal ions in the iron group in the periodical table are the most likely to interfere. However, for the samples in question the concentration levels of these metal ions should be far below any interfering level. Since Ferrozine reacts with Fe^{2+} but not with Fe^{3+} a reduction step is required. Hydroxylamine hydrochloride is selected for this purpose.

Polyphosphates in the sample matrix might complex with iron. The samples are therefore pretreated with hydrochloric acid. In some cases even boiling of the acidified sample or digestion with potassium peroxidisulfate might be necessary.

Step 3.   Ferrozine seems to fulfill the working range and sensitivity requirements. Selectivity for Fe^{2+} is also promising. The reagents are commercially available (Ferrozine and hydroxylamine hydrochloride).

Step 4.   Maximum sensitivity is strived for, i.e. the dispersion coefficient should be close to 1. The carrier flow rate should thus be high while the reagent flow rates should be kept low. The sample volume should be large, at least 200 μl. Fig. 8.1 shows the suggested manifold.
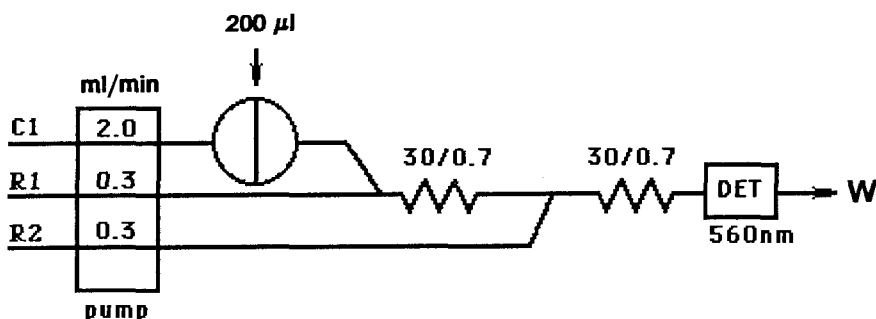
Fig. 8.1. Theoretical manifold design for determination of iron. R1 = combined reagent of Ferrozine and hydroxylamine hydrochloride and R2 = buffer solution (10% sodium acetate). Numbers at coils denote length/i.d. (cm/mm).

The calculated dispersion coefficient for this FIA system is about 1.3 provided that a large sample volume (≥ 200 μl) is injected. Even lower dispersion coefficients can be achieved by increasing the carrier flow rate further and by decreasing the reagent flow rates at the same time. However, this could lead to back pressure in the system with an insufficient reagent supply as a consequence. Poor reproducibility would then result. By increasing the injected sample volume above 200 μl very little is gained. On the contrary, peak broadening occurs and the sampling frequency drops drastically.

Some calculations of the necessary reagent concentration can now be made. At the upper concentration level (1 mg/l) R1 dilutes the injected sample by a factor of 2/(2 + 0.3) derived from flow rates. The resulting concentration, 0.870 mg/l, corresponds to a 0.0156 mM Fe solution. The reagent concentration is assumed to be y mM. In this case the carrier dilutes the reagent R1 by a factor of 0.3/(2 + 0.3) corresponding to a concentration of 0.130 y mM at the first confluence point. Each ferrous ion binds three Ferrozine molecules. Assuming further that a 5-fold reagent excess is satisfactory, then 0.130 y = 3 * 5 * 0.0156 and y = 1.80 mM which corresponds to 0.926 mg/l of Ferrozine (MW: 514.36). So R1 is composed of 0.926 mg Ferrozine and 35 g hydroxylamine hydrochloride per liter.

Step 5. Baseline seems to be steady. The dispersion coefficient turned out to be 1.11 instead of the "theoretical" value of 1.3 for a 200 μl sample. This difference can be due to different aging of pump tubes and to back pressure in the system. The detector response was good when a standard was injected but an appreciable matrix effect (negative and positive deflections before and after the peak) appeared, see Fig. 8.2.
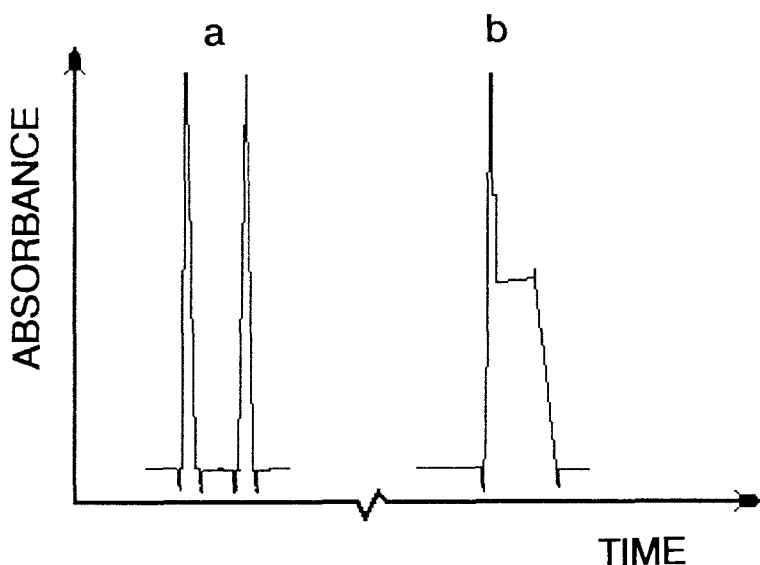
Figure 8.2. Detector response obtained with the initial manifold for iron. 200 μl of a 200 $\mu g/l$ Fe^{3+} standard solution was injected (a). A stopped-flow response curve for the same standard (b).

The magnitude of the obtained signal, about 0.1 A.U., corresponds roughly to the theoretical batch value which can be calculated using the molar absorptivity value of 27,900. The pH value of the waste line was 4.5 indicating that the buffering capacity of the R2 solution was adequate. The absorbance of the iron-Ferrozine complex showed a maximum at 560 nm, see Fig. 8.3.
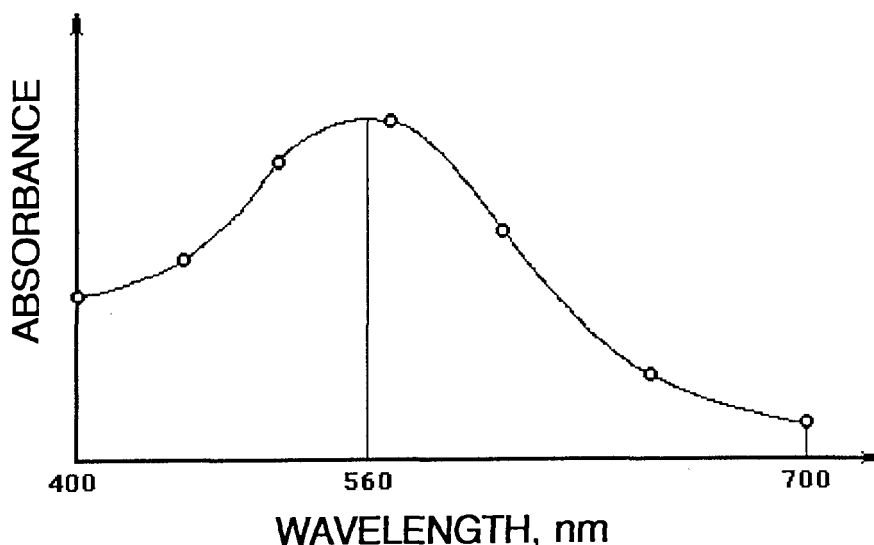
Fig. 8.3. Absorbance spectrum for the iron-Ferrozine complex at pH 4.5.

As already mentioned, the peak shape is distorted due to influence from the sample matrix. This can probably be remedied by changing the carrier stream composition. Thus, some further experiments are needed. At this point it cannot be concluded that the mixing is complete but the total length of mixing coils in the system should be sufficient. However, it is too early to be absolutely positive about that. Different stopped-flow experiments showed that the reaction was fast and almost complete, see Fig. 8.2b. Several experiments were performed with stops made at different times meaning that different sections of the sample gradient were trapped in the flow cell for the purpose of reaction rate observations. In this way a large variety of sample/reagent concentration ratios was created. The reaction was fast in all cases so it can be concluded that the original reagent excess (5-fold) was quite satisfactory.

Step 6.   Different pH values in the resulting waste line were created by changing the composition of the R2 solution (by addition of HCl or NaOH). The pH "window" was found to be 3.6 - 6.0.

Step 7.   The following peak height values were obtained at different temperatures:

Temperature	A.U.
14 °C	0.399
24 °C	0.417
35 °C	0.449
48 °C	0.470

The immediate conclusion is that rather little is to be gained by raising the temperature. The gain is probably outbalanced by the disadvantages resulting at elevated temperatures: air bubbles released in carrier and reagent solutions, slow attainment of a constant temperature value, extra accessory needed (thermostat) etc. Some stopped-flow experiments were also made at the different temperatures. No significant absorbance increases resulted during the stop periods. In fact, the reaction had reached at least 90% of the "steady state value" within 20 seconds.

Step 8.   A calibration test of the designed manifold displayed linearity over the range 0.025 - 0.90 mg Fe^{3+}/l. However, the matrix effects made the peak height evaluation imprecise especially at lower concentrations. These effects are often rather pronounced in low dispersion manifolds and when highly concentrated reagents are used. The carrier flow is temporarily interrupted during the turn of the injection valve while the reagent flows are not. Local excesses of reagent are then created in the united flow. The "ghost" peaks occur as a result of changes in refractive index caused by the local reagent excesses. One way to circumvent this problem would be to use less concentrated reagents at higher flow rates. However, this will lead to an increase in the sample dispersion which is synonymous with a decreased sensitivity. The first step must be elimination of the matrix effects without paying the price of a significant decrease is sensitivity. There are two possibilities, either to use an injection valve with a by-pass or to use two carrier streams, see Fig. 8.4.
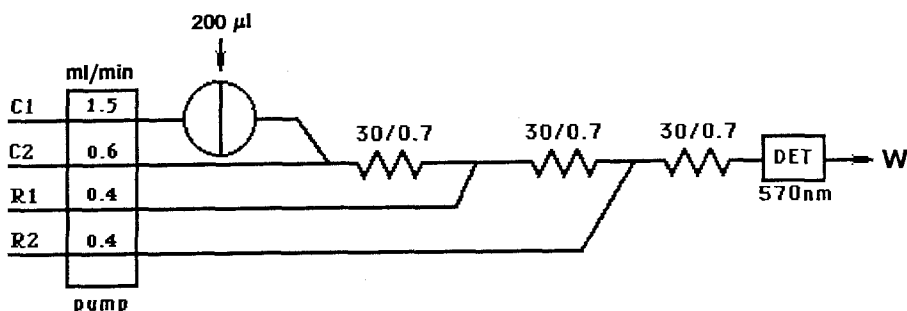
Fig. 8.4. Determination of iron with the Ferrozine method. Manifold with two carrier streams to avoid matrix effects. Numbers above coils denote coil length/i.d. (cm/mm). For details, see text.

The second carrier flows continuously and is not influenced by the valve operations. The manifold in Fig. 8.4 yielded a dispersion coefficient of about 1.5 which resulted in some loss in sensitivity in comparison with the original manifold (D = 1.1). On the other hand, the matrix effects disappeared and very reproducible results were obtained. The calibration graph was linear over the range 0.025 to 0.8 mg Fe/l. The sampling frequency was about 80/h for an injection volume of 200 μl. It has already been shown that the chemical reaction for the method is extremely fast. Thus, very little is probably gained by increasing the coil lengths. A coil diameter of 0.7 mm was preferred instead of the usual 0.5 mm since improved mixing was obtained. The steady baseline obtained for 0.7 mm i.d. coils was interpreted as proof of better mixing. The Ferrozine concentration was decreased from 0.93 to 0.75g/l and the sodium acetate solution (R2) was diluted from 10% to 8%. The sample throughput was studied for different injection volumes, see Fig. 8.5.
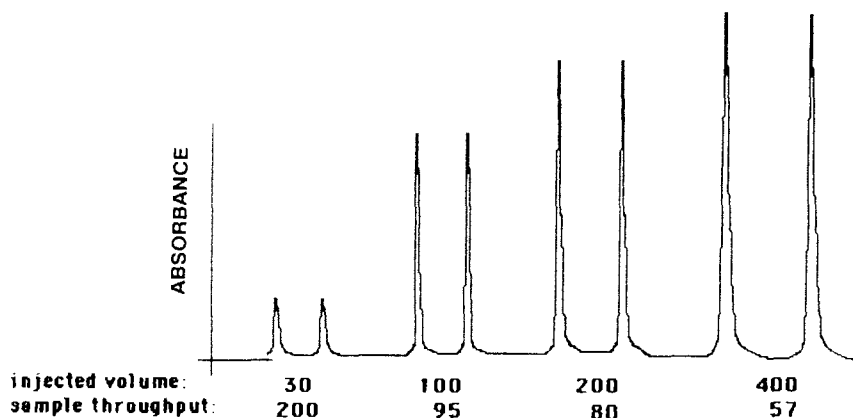
Fig. 8.5. Detector response (duplicate injections) and sample throughput as a function of injection volume. S = 0.5 μg Fe/l. Manifold: see Fig. 8.4.

As a result of this study the injection volume of 200 μl was selected as a compromise between sensitivity and sample throughput.

Step 9.   Interference studies were performed for chloride, sulfate, sodium, calcium, manganese and phosphate ions present at common concentration levels in typical sample matrices. Deviations in signal response were less than 2% for all these ions.

Step 10.   Method validation is an important step in all application development. It is applied not only for FIA methods but for all developed analytical methods. Consequently, it will not be treated in any detail here.

8.4   FAULTFINDING DURING METHOD DEVELOPMENT

Many apparent malfunctions of an FIA system can be traced to the chemistry of the analytical method rather than to shortcomings of the hardware. This means that the essential first step of faultfinding must be to find out if the problem has a chemical or a mechanical origin (or both). If the intended analytical method is adapted to FIA for the first time it is good practice to check the chemistry manually in a batch procedure prior to the very first injection of standard solution. Sample and reagent volume proportions should then be the same as those applied later on in the FIA system. The hardware can

be tested separately by injection of a dye solution in the case of spectrophotometric detection. The dye solution is also helpful in that it can be used to check the flows in different streams as well as to check the function of the injector. PTFE tubes are not particularly transparent but a dark-blue dye inside almost any tube can be readily distinguished.

In summary, check the chemistry first. Use the check list below. Then proceed with the tests of the hardware.

Check list, chemistry

Step 1.   Mix the standard/sample with the reagent(s) in proportions corresponding to those intended to be applied in the FIA system. Use the flow rate values according to the color codes of the pump tubes. Note that nominal and real flow rates can differ, so to be quite sure the individual flow rates should be measured by pumping water out of a vessel with a known volume during a known time.

Step 2.   Inject or pump the batch mixture into the FIA system (using distilled water as carrier). If no signal response is obtained then check carefully:
- the detector itself (see check list, hardware). Use a second detector, if available, to verify the detector malfunction
- the reagent used to prepare the reagent solution. Note that many indicator names are almost identical (o-cresolphtalein and o-cresolphtalein complexone are two quite different reagents)
- the concentration levels of sample and reagent solutions.
  It is a common mistake to mix up g and mg...
- the pH of the standard/sample solution, the pH of the reagent(s),
  the pH of the resulting mixture leaving the FIA detector.

Check list, hardware

Hardware malfunctions can be divided into four main categories according to their origin: liquid delivery, injection valve, manifold and detector malfunctions.

A.   Liquid delivery malfunctions

Check each individual flow by pumping water and
      - replace flat or misused pump tubes
      - lubricate pump tubes with silicone oil

- adjust compression cam/band tension
- disconnect the injector and the manifold.

Is the liquid delivery OK now? If yes, connect injector, check according to scheme below. If no, continue to try to remedy the problem. See Chapter 3.4.

Note that the flow rates measured individually with no injector or manifold connected will change when all flows are brought together and passed through the injector/manifold. Problems with the flow dynamics in the system may arise at this point if high flow rates are combined with low flow rates. The individual flow rates will also change significantly if a narrow-bore manifold is connected. The resulting back-pressure could even give rise to ceasing or reversing flows! A redesign of the manifold might become necessary if the expected flow conditions cannot be accomplished.

B.   Injection valve malfunctions

Use a dye solution when checking
- the aspiration flow rate

If the stream from the injector to waste is air-segmented then check and tighten the connections or lower the aspiration flow rate. Air bubbles may be formed in the sample stream due to underpressure even in a completely tight system
- the sample loop

If the sample loop is contaminated by grease or dirt then air bubbles easily lodge in the loop. Clean or replace the injection loop
- the by-pass loop

The sample may leak into the by-pass so that it is partially filled during the sample aspiration cycle. Fill the by-pass with air or dye and check if leaking really occurs. Check both valve modes: fill and inject. The i.d. of the by-pass tube should be smaller than the i.d. of the main conduit to the injection valve, see Chapter 3.6.
- activation of the injector

Is the valve turning correctly and completely? Is it returning to the fill position at the desired time? Visually observe the injected dye slug in the carrier. This test can also be made by injecting air into the carrier. The resulting air segment should not be segmented with liquid.
- the carrier flow rate

Is the real flow rate close to the expected flow rate? Is the flow rate changed when the valve is activated, i.e. when it turns from the fill position to the injection position?

C.   Manifold malfunctions

Pump water through the manifold. Are the flow channels in the manifold blocked? Remove all mixing coils, visually check all channels and bores. Use nylon thread (not metal wires) to clean the bores. Connect the mixing coils one by one and check in between all connections to make sure that the liquid flows in a normal manner.

Tubing constriction points and material flakes or pieces in the manifold channels should definitely be avoided.

Special manifolds were described in Chapter 3. Each type of manifold requires its own faultfinding scheme. It is beyond the scope of this chapter to present faultfinding schemes for all manifold types.

D.   Detector malfunctions

Spectrophotometers:
        - baseline drift
Allow some time, minimum 30 minutes, for the detector to warm up.
Check if air bubbles accumulate in the flow cell.
Degas carrier and reagent solutions.
Check the stability of the light source and of the photodiode, photomultipliers, etc.
Are the reagents degrading?
        - background absorbance too high
Check if the flow cell is contaminated. Clean the external walls of the flow cell with tissue paper soaked in acetone or absolute ethanol.
Clean the internal parts of the flow cell by rinsing with 5 M sodium hydroxide. Use gloves and safety glasses.
Rinse with large amounts of distilled water (or weakly acidic solution).
Have the reagent solutions become discolored?
Is the wavelength setting correct? Perform a scan.
        - precipitation formation in the flow cell
Can the concentration levels of the reagents be decreased?
Is it possible to intermittently add a rinse solution (e.g. acid, base, EDTA) to dissolve the precipitation?
        - no signal change observed upon sample injection
Is the chemistry working: pH, correct reagents, etc.?
Is the recorder connection correct?
Does the detector respond at all? Block the flow path with a paper strip; this should result in a maximum deflection.

Is the wavelength setting correct?

A coarse visual test of the wavelength setting can now be made by observing the projected light beam on the paper strip. Set wavelength to 540 nm - green color. Set wavelength to 633 nm - red color (common display color). Set wavelength to 586 nm. At this wavelength the light is changing from yellow to red. Set wavelength to 576 nm - bright yellow color. Set wavelength to 596 nm - distinctly red color. This means that the monochromator can be adjusted with an accuracy of ±10 nm using this simple test.

Is the dark current adjustment correct?

    - signal changes too small

Check attenuation.

Mix sample and reagents in a beaker in the same proportions as in the FIA system. Pump the mixture through the flow cell.

Is the signal level as expected?

Are the reagent concentrations sufficiently high?

Is the reaction rate too slow? Perform stopped-flow experiments to check this.

Is the dark current adjustment correct?

The three most common reasons for too small peaks are: wrong pH value of the sample/reagent zone, incorrect wavelength setting, and incorrectly prepared standards/samples.

Hopefully this chapter gives you a blueprint to following when carrying out method development. In the large majority of the cases, the FIA system will make the batch chemistry, which is now automated, more precise and reliable. However, the analyst's mind should not be limited to existing chemistries. Combinations of selectivity enhancement techniques and the unique features of FIA provide the opportunity to create new methods. Even reagents that were discarded because they were "unstable under batch conditions" might exhibit excellent selectivity for specific analytes and be very reproducible under FIA conditions. Imagination and chemical intuition can create exciting new methods!

REFERENCES

1.   D. Betteridge, T.J. Sly, A.P. Wade and D.G. Porter, Anal. Chem., 58 (1986) 2258. [823]

2.   J. Moller, in Analytiker Taschenbuch, Band 7, Springer-Verlag, Berlin, 1988, p. 199-275. ISBN 3-540-17689-6 (in German). [-]

CHAPTER 9

WATER, AGRICULTURE, SOIL AND ENVIRONMENTAL

9.1 WHY FIA, WHEN FIA FOR THESE SAMPLES

For these types of analytes/samples the primary advantage of FIA is economic. A secondary advantage is ease of method development or, more often, method refinement. Central to both advantages is the acceptance of an automated form of the batch "Standard Methods". The issues concerning acceptance of automated forms of the Standard Methods should be rather minimal. This is because the FIA system is utilizing the accepted chemistry of the Standard Method. Sample and reagent concentrations can be adjusted to conform with the batch conditions. Because of the reproducible timing and controllable sample dispersion the FIA system simply improves the precision of the batch method. The increase in sample throughput is an added economic benefit. FIA methods have detection limits comparable to batch methods. The detection limits cannot be dramatically improved without significant modifications in the method.

The complete FIA system would contain a sampler, the FIA unit, detector, and recording devices which are capable of sample identification and evaluation of the detector signal in terms of analyte concentration. One economic advantage of this type of system is that if the laboratory demands do not justify dedication of the FIA system to a particular determination, this system can be easily and rapidly modified to carry out a different method. In terms of laboratory demand the rapid method changing capability of FIA would allow a small or medium laboratory which cannot afford dedicated or specific instruments for particular methods to have the capability of performing most methods on short notice. Larger laboratories can still benefit from the high throughput of FIA even when it is used as a dedicated system. The larger laboratory would also have the flexibility of using an FIA system to do the occasional method by FIA.

Soil laboratories have extremely busy schedules in the planting season. If the laboratory has dedicated instruments, they will be utilized sparingly during the off-seasons while FIA instruments could be used for other projects in the off-season. Secondly, the FIA system is more suited for excessive workloads than most dedicated instruments. The only routinely replaced part of an FIA system is the pump tubes. The FIA system is rugged!

A secondary benefit for these types of laboratories is the capability of FIA to modify the sample matrix. Modification can be dilution, analyte transplantation (i.e. dialysis, gas diffusion, liquid-liquid extraction, ion-exchange) and filtration (i.e. particle filtration, column

ion/molecule trapping). By modifying the sample matrix several benefits can be realized. First, the detector performance can be improved. For example in spectrophotometry, removal of constituents which create baseline noise improves detectability of the analyte. Second, interferents can be minimized or eliminated resulting in selectivity enhancement. Third, preconcentration of the analyte allows for lower overall detection limits. Fourth, the reagent/analyte chemistry can be carried out under "optimized" conditions. This is important for those methods in which the reaction is not 100% complete during the observation.

Clearly, FIA has some outstanding advantages for the water, agricultural, soil and environmental laboratories. However, just as with any chemical system, the chemist must be aware that in flow through detectors where a change in a physical property, i.e. ionic strength and viscosity, will change the signal, some type of matrix matching must be done. Matrix matching in FIA means, in most cases, adaptation of the carrier stream composition so that it matches the sample matrix. For instance, an artifical seawater solution is used as carrier when seawater samples are to be injected.

Several specific examples of determinations made in the field of water analysis will now be presented. These examples have been chosen for one of two reasons: 1) the analyte is very important as evidenced by its large number of determinations made per year, like phosphate, or 2) the method demonstrates a particularly useful FIA technique. Obviously, all examples cannot be covered, therefore, the reader is referred to the extensive index for the bibliography.

9.2 PHOSPHATE (ORTHOPHOSPHATE, PHOSPHORUS)

Phosphate is one of the most frequently performed determinations in a water analysis laboratory. Several chemical principles and sample pretreatment procedures are applied (1-4). The low concentration levels of phosphate requires special precautionary measures with respect to sample handling and maintenance of equipment. Matrix matching is necessary for pretreated samples. Precipitation and adsorption of the colored complex may occur in the flow conduits.

Sample pretreatment and definition of the analyte

Depending on the sample pretreatment different forms of phosphorus compounds can be determined in a water sample. Phosphate is sometimes carelessly used without any further specification of the analyte. The analytical methods are all based on specific reactions between orthophosphate in the sample and consitutents in the added reagents.

The color formed is measured in a spectrophotometer. All chemical reactions applied for phosphate prescribe acidic conditions. This means that all phosphorus compounds, e.g. polyphosphates, which are readily hydrolyzed to orthophosphate at low pH values, will be included in the determination.

Water samples are usually preserved by addition of sulfuric acid and stored in the dark at 4 °C. The final concentration of acid is typically 0.04 M. Sometimes the samples are filtered. The filtration should be made immediately after the samples have been taken and before addition of the preserving acid. A 0.45 μm membrane filter is used. Phosphate results obtained for samples treated in this way are usually referred to as **dissolved inorganic phosphorus.**

Total phosphorus is determined in non-filtered, acid preserved samples after digestion with peroxodisulfate. Potassium peroxodisulfate, 0.5 g, is added to 50 ml of the sample. This solution is boiled cautiously until a volume of 5 - 7 ml remains. Alternatively, the solution is heated in an autoclave or pressure boiler at 150 - 200 kPa. In either case the residue is diluted to a known volume with distilled water before analysis.

<u>Chemical methods</u>

(A)   Orthophosphate, originally present in the sample or formed through hydrolysis, reacts with ammonium molybdate to form a heteropoly molybdophosphoric acid compound. This is reduced with stannous chloride in an aqueous sulfuric acid medium to form molybdenum blue. The molybdenum blue color is measured at 650 - 700 or at 880 nm.

(B)   Orthophosphate, originally present in the sample or formed through hydrolysis, reacts with ammonium molybdate and potassium antimonyl tartrate to form an antimony-phospho-molybdate complex. This complex is reduced to a blue colored complex by ascorbic acid and measured at 650 700 or 880 nm in a spectrophotometer.

(C)   Orthophosphate, originally present in the sample or formed through hydrolysis, reacts in an acidic solution with ammonium molybdate and ammonium vanadate to form a yellow colored phospho-vanado-molybdate complex which is measured at 410 nm.

These are the three main methods for "phosphate" but a large number of different versions of these methods exist.

FIA principles for phosphate determination

Method A:

The manifold is shown in Fig. 9.1. The sample, S, is injected into a carrier stream, C1, and merged with a second carrier, C2. Using this arrangement, matrix effects caused by temporary fluctuations in the flow rate can be minimized, see Chapter 8.3, step 8. For example, such fluctuations occur when the injection valve is turned. Ammonium molybdate solution, R1, and stannous chloride solution, R2, are merged with the joint carrier.
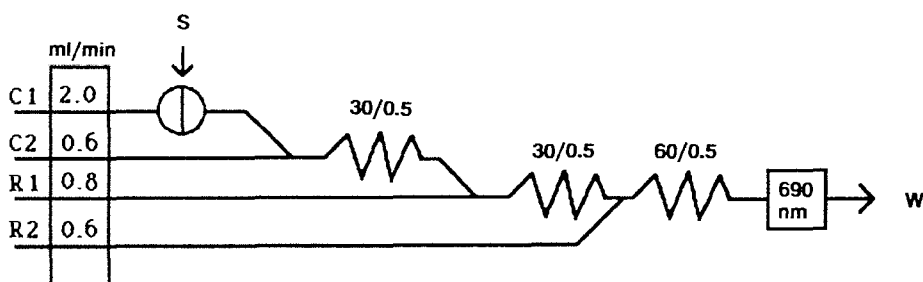

Fig. 9.1. Manifold for the determination of phosphate according to the molybdenum blue stannous chloride method. Numbers above coils denote coil length/i.d. (cm/mm).

The chemical reaction between the analyte and the reagents is fast. The analysis can be performed at room temperature. The absorbance spectrum shows two almost equally useful regions, at 650 - 700 and 880 nm.

The carrier streams, C1 and C2, are adapted to the sample matrix so that distilled water is used for nonpreserved samples and sulfuric acid for digested and preserved samples. The acid concentration of the carrier and of the standard solutions should match that of the samples.

Reagents

R1   Dissolve 5 g ammonium molybdate in 300 ml distilled water. Add slowly and cautiously, with stirring, 17.5 ml concentrated sulfuric acid and dilute to 500 ml with distilled water. Degas this solution. R1 is stable for several months.

R2   Add slowly and cautiously, with stirring and cooling, 14 ml concentrated sulfuric acid to 300 ml distilled water. Dissolve 0.1 g stannous chloride and 1 g hydrazinium chloride and dilute to 500 ml with distilled water. Degas this solution. R2 is stable for at least 1 week if stored at 4 °C.

Using an injection volume of 200 μl for maximum sensitivity, the detection limit depends mainly on the detector but should be in the range of 1 - 10 μg/l P. The flow rate scheme in Fig. 9.1 may be modified and improved depending on individual properties of pumps, injector and detector. The upper limit for this system is about 500 μg/l. For concentrations larger than 500 μg/l use a smaller injection volume while reducing the flow rate of C1 and increase all other flow rates.

There are some interferences for this method. Arsenic as arsenate at 100 μg/l gives a response equivalent to about 30 μg/l P. If arsenate is present in the sample, sodium metabisulfite and sodium thiosulfate can be added to the acidic sample (sulfuric acid) before addition of ammonium molybdate and stannous chloride. Sulfide can be present in the sample at a concentration level of up to 2 mg/l. It can be removed by purging the acidified samples with nitrogen gas. Silicate can be present at levels up to 5 mg/l. Silicate reacts slowly with molybdate; consequently this reaction is kinetically discriminated in an FIA system. A lot of other species may interfere at levels not found in normal water samples, for example selenium, copper, chromium, vanadium, and fluoride. By standard addition of known amounts of phosphate to a sample, presence of such interfering species can be ascertained.

Method B:

The principles for this method are essentially the same as for the molybdenum blue-stannous chloride method. The compositions of the reagents R1 and R2 differ. The manifold should be termostatted to 40 °C to obtain a fast chemical reaction and thereby enhanced signals for the injected samples. Details about matrix matching, working range and interferences are found in the previous method description. The absorbance spectrum shows two useful regions, one at 650 - 700 and the other one at 880 nm. The absorbance value at 880 nm is about 1.5 times larger than the corresponding absorbance value at 650 - 700 nm.

Reagents

R1   Dissolve 5 g ascorbic acid in 500 ml distilled water. Degas this solution. R1 is stable for at least one week if it is stored in an amber bottle at 4 °C.

R2   This reagent, usually called *molybdate solution*, is composed of three individually prepared solutions, S1 - S3. S1: Carefully add 120 ml concentrated sulfuric acid to 170 ml distilled water in a beaker and cool. S2: Dissolve 13 g ammonium molybdate in 100 ml distilled water. S3: Dissolve 0.35 g potassium antimonyl tartrate in 100 ml distilled water. Mix all three, S1 - S3, and degas the solution. R2 is stable for about 2 months if stored in an amber glass bottle at 4 °C.

Depending on the sample matrices and on the flow cell configuration it might be necessary to use reagents which are more dilute than the R1 and R2 reagents described above. Concentrated reagents might give rise to refractive index phenomena (matrix effects). In some manual procedures for phosphate, R1 and R2 are combined to one single reagent solution. This solution has a limited stability, maximum 8 hours. Although no comprehensive report is available this approach might be considered also for an FIA procedure.

Method C:

The sample, S, is injected into a carrier stream, C1, and merged with a second carrier, C2, to minimize "matrix effects", see Chapter 8.3, step 8. An acidic vanadate-molybdate reagent solution, R1, is merged with the joint carrier stream, see Fig. 9.2.
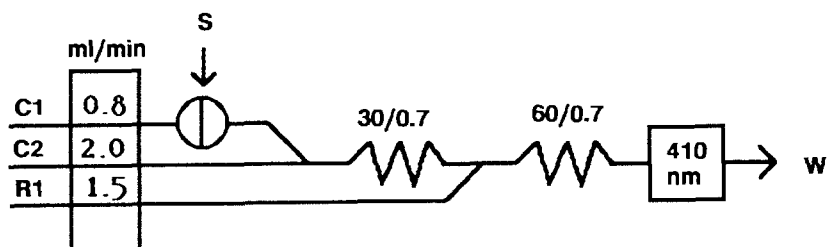

Fig. 9.2. Manifold for the determination of phosphate according to the vanadomolybdate (yellow) method. Numbers above coils denote coil length/i.d. (cm/mm).

The chemical reaction between the analyte and the reagent is fast. The analysis can be performed at room temperature. The absorbance values are continuously increasing in the range 450 - 400 nm ($A_{400} > A_{450}$). The carrier streams, C1 and C2, and the standard solutions used are adapted to the sample matrix composition.

Reagent

R1   This reagent is composed of three individually prepared solutions, S1 - S3.
S1: Dissolve 40 g ammonium molybdate in 100 ml distilled water. Heat, if necessary. S2: Dissolve 2 g ammonium vanadate in 100 ml distilled water. Heat, if necessary. S3: Add 135 ml nitric acid (65%) to 100 ml distilled water. Add S2 to S3 while stirring. Slowly add S1 to the S2 and S3 solution. Dilute to a final volume of 500 ml with distilled water. A slight precipitate may be formed with time. Degas and filter R1 before use. R1 is stable for several months.

Working range

The working range is 2 - 500 mg/l P depending on the sample volume injected and the flow rates of C1, C2 and R1. By selecting a wavelength, e.g. 460 nm, at which a lower absorbance is obtained for the phospho-vanado-molybdate complex, the working range is further extended.

Standard stock solution, 100 mg P/liter

Dissolve 0.4393 g anhydrous potassium dihydrogen phosphate, dried for 2 hours at 80 °C, in distilled water and dilute to 1 liter. Store in an amber borosilicate glass bottle at 4 °C.

Remarks

Phosphate is a common contaminate in a laboratory which means that beakers, bottles, flasks, etc., used for phosphate samples and reagents should not be used for any other purpose. Sample bottles should be made of borosilicate glass or polyethylene. Phosphate adsorbs on the walls so acid washing is necessary. Wash with hot 1:1 HCl and rinse with distilled water. The acid-washed glassware should be filled with distilled water and treated with all the reagents to remove the last traces of phosphorous. Commercial detergents should never be used. Store the vessels protected from dust. Molybdenum "blue" and "yellow" species may stick to the manifold walls and in the flow cell. At high phosphate concentrations precipitates may even appear in the flow system giving rise to

a positive baseline drift. The precipitate can be dissolved by pumping diluted ammonium solution (1:10). This washing could be included as a mandatory step in the closing down procedure.

9.3   NITRATE (TOTAL NITROGEN)

Nitrate is a commonly performed determination in a water analysis laboratory. The standard method prescribes reduction of nitrate to nitrite using a reduction column (5-8). The sum of nitrate and nitrite originally present in the sample is determined spectrophotometrically. The originally present nitrite has to be determined separately and subtracted from the sum to obtain the nitrate concentration. Nitrate can also be determined potentiometrically using a nitrate selective electrode.

The nitrate samples should be either free from turbidity or should be filtered through a 0.45 μm membrane filter. No preservation is recommended. The samples should be stored in the dark at 4 $^\circ$C and be analyzed within three days after collection.

The total nitrogen samples are digested in the presence of alkaline peroxodisulfate. The nitrogen compounds are then converted to nitrate. The procedure is as follows: 20 ml of the sample and 10 ml of a 1% peroxodisulfate solution (dissolved in 0.12 M NaOH) are mixed and treated in an autoclave at 120 $^\circ$C and 200 kPa for 30 minutes. After cooling to room temperature an aliquot of 1 ml 0.5 M sulfuric acid is added. The digested solution is transferred to a 50 ml measuring cylinder. The digestion tube is washed with a small portion of distilled water and this portion is also transferred to the measuring cylinder. One drop of phenolphthalein solution is added and the mixture is "titrated" with 0.12 M NaOH until a pale pink color is obtained. 1 ml of a 5 M ammonium chloride solution is added and finally distilled water up to a total volume of 50 ml.

Chemical principles for the nitrate methods

(A)   Photometric method.

Nitrate is reduced to nitrite by passing the sample through a cadmium reductor. Nitrite, formed in the reduction column or present originally, reacts with sulfanilamide in an acidic solution. A diazo compound is formed. The diazotized reaction product is then coupled with N-(1-napthyl)-ethylenediamine dihydrochloride to form an azo dye the absorbance of which is measured at 540 nm. The evaluated absorbance value corresponds to the sum of nitrite and nitrate in the original sample. In a second determination

the nitrite concentration is obtained using the same chemical principles but with the reduction step omitted.

(B)   Potentiometric method.

A sample aliquot is brought to the surface of nitrate selective electrode in an FIA system. A reference electrode is situated in the same system. The potential difference is measured.

FIA principles for nitrate determination

Method A (photometric method):

Manifold

The sample, S, containing nitrate and nitrite is injected into a carrier stream, C. The nitrate is reduced to nitrite in the cadmium reductor. An acidic sulfanilamide solution, R1, is added first followed by the addition of N-(naphthyl)-ethylenediamine solution, R2. The sample and reagent mixture is brought to a flow cell for absorbance measurement at 540 nm, see Fig. 9.3.
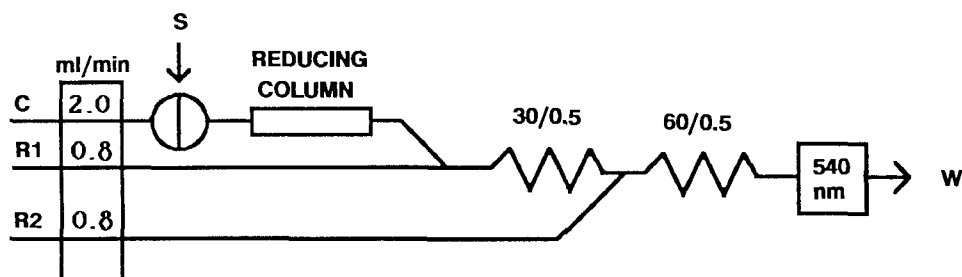

Fig. 9.3. Manifold for the determination of the sum of nitrate and nitrite using an in-line reduction column. Numbers above coils denote coil length/i.d. (cm/mm).

The same manifold but with the cadmium reduction column removed or by-passed is used for the determination of nitrite originally present in the sample.

Column preparation

Several preparation principles for the cadmium reductor are applied. "Off-line" reduction columns are usually filled with sieved cadmium granules (0.5 - 1.0 mm) and treated with 1% mercuric chloride solution. Commercially available in-line reductors either consist of a short mixing coil packed with cadmium filings mesh 20 (Alpkem or Technicon) or a plastic tube filled with copperized cadmium granules, 0.2 - 2.0 mm (Tecator).

The preparation of copperized cadmium reductor is taken as an example. 5 - 10 g of cadmium granules are swirled with 0.5 M HCl for about one minute. The granules are then swirled in a 1% solution of copper sulphate and finally rinsed with 0.1 M NH_4Cl solution. From now on it is very important that the cadmium granules are not allowed to dry. Fill the column first sealed in one end with glass-wool or cigarette filter. Make sure that the packing is efficient. No air pockets should be present. Seal the other end of the column. Pump carrier solution, 0.1 M NH_4Cl, through the column. If necessary, tap the column to release trapped air. Cadmium is poisonous - handle granules and solutions with care.

Matrix matching

The carrier stream, C, consists normally of a 0.1 M ammonium chloride solution. When total nitrogen is determined in a water sample the carrier stream has to match the digested sample matrix. Carrier is then prepared as follows: Prepare an oxidation solution by dissolving 10 g potassium peroxodisulfate in 1 liter of 0.12 M NaOH. Add 200 ml of this solution to 400 ml distilled water. Boil the mixture on a heating plate for about 30 minutes. Cool and add 20 ml 0.5 M sulfuric acid. Add one drop of phenolphthalein indicator solution and "titrate" with 0.12 M NaOH until a pale pink color has been obtained. This C solution is stable for several months.

Reagents

R1   Dissolve 5 g sulfanilamide in a mixture of 26 ml concentrated hydrochloric acid and 300 ml distilled water. Fill to 500 ml with distilled water. Filter and degas. This solution is stable for several months if stored in an amber glass bottle.

R2   Dissolve 0.5 g N-(1-naphthyl)-ethylenediamine dihydrochloride in 500 ml distilled water. Filter and degas. This solution is stable about one month if stored in an

amber glass bottle at 4 °C. Discard this reagent if it has is discolored. Avoid skin contact.

Working range

The working range is 0.1 - 2 mg/l NO_3-N (nitrate nitrogen) using an injection volume of 200 μl and 0.5 - 5.0 mg/l NO_3-N using 40 μl. The working range can be extended up to *40 mg/l* if a stream of distilled water is merged with the carrier stream at a site after the reduction column. Zone sampling and sample splitting can also be applied.

Interferences

High concentrations of aromatic amines, copper, iodide and humic acids will interfere. The pH value of the carrier and reagent streams should be kept constant during a set of analysis. Colored constituents in the sample which absorb in the photometric range 500 - 600 nm will interfere. The sample must not contain strong oxidants or reductants.

Method B (potentiometric method):

Manifold

The sample, S, containing nitrate is injected into a carrier stream and brought undiluted to the surface of a nitrate selective electrode arranged in an FIA system. The reference electrode can be a calomel electrode placed in the flow system not too far away from the nitrate electrode. Flow cells for potentiometry with ion-selective electrodes are available from almost all FIA manufacturers.

Reagents

The carrier should preferably contain a low concentration of nitrate, e.g. 10^{-5} M. The working range limits are set by the performance characteristics of the nitrate selective electrode but are typically 10^{-1} - 10^{-5} M.

Interferences

Several anions will interfere, in particular perchlorate, iodide, chlorate, bromide and nitrite. Also, chloride interferes at the low nitrate levels.

<u>Standard stock solution, 1 g N/liter</u>

Dissolve 3.034 g sodium nitrate (or 3.609 g potassium nitrate) in 500 ml distilled water. Store in an amber bottle.

9.4   AMMONIA (AMMONIA NITROGEN, AMMONIUM)

Along with phosphate and nitrate/nitrite ions, ammonia is on the list of more or less compulsory species to be determined in all kinds of water samples. The standard method is the classical indophenol blue reaction (9-12). Ammonia reacts with hypochlorite to form monochloramine which in turn reacts with phenol to form indophenol blue. Nitroprusside ions are sometimes used as a catalyst. In an alternative approach, the chlorinated ammonia is determined with o-tolidine replacing phenol. Since the above mentioned procedure uses chemicals which, in various degrees of seriousness, can pose hazardous effects to the analyst, an alternative FIA method based on gas diffusion has been developed. This method is gaining a widespread acceptance also because of the fact that colored species present in the sample do not interfere.   The method accepts large variations in the sample matrix.

<u>Sample pretreatment</u>

Collected water sample should be analyzed the same day. The aliquot used for analysis is obtained by decantation. Storage of samples in open vessels during the work should be avoided due to the large contamination risk. Samples with visible turbidity should be centrifuged if the indophenol blue method is applied but need no pretreatment if the gas diffusion method is applied.

<u>Chemical principles</u>

(A)   The indophenol blue method

In an alkaline solution, pH 10.8 - 11.4, ammonia reacts quantitatively with hypochlorite forming monochloramine. This latter compound reacts with phenol in the presence of catalytic amounts of nitroprusside ions and in the presence of excess amounts of hypochlorite to form indophenol blue. The exact reaction mechanism is not fully understood. The amount of indophenol

blue formed is measured spectrophotometrically at 630 nm.

(B)   The o-tolidine method.

Due to the carcinogenic properties of o-tolidine this method is not recommended in any way including FIA.

(C)   The gas diffusion method.

Ammonium ions in the sample are converted to ammonia gas in the flow system by addition of sodium hydroxide. The ammonia gas diffuses through a gas permeable membrane and into a recipient stream comprised of an acid-base indicator. The color change caused by the ammonia gas is measured. The chemical principle is thus rather simple. The analyte, the ammonium ion, is transformed to ammonia gas and transplanted into a new matrix. The original sample matrix composition is therefore not critical for the analytical results as long as the conversion to ammonia gas is complete. The new matrix comprises an acid-base indicator which can be buffered to decrease the sensitivity and to extend the working range of the method. A certain amount of a strong acid, e.g. hydrochloric acid, can also be added. In this case, the color shift of the indicator does not occur until all protons originating from the strong acid have been neutralized by ammonia. At this point a relatively small increase of diffused ammonia gas will cause a large change in pH and color of the indicator stream. Thus, by adjusting the amount of strong acid the sensitive working range can be displaced - a sort of chemical zero suppression is obtained.

Several acid-base indicators have been used to compose the ammonia recipient stream. Phenol red gives an acceptable working range but due to the limited solubility of this indicator in water adsorption onto the manifold and the glass flow cell may occur. The indicator mixture developed by Tecator covers a large working range with maintained sensitivity. Calibration graphs are not linear which is easily explained by the equilibrium reaction

$$HIn + NH_3 \rightleftharpoons NH_4^+ + In^-$$

At high levels of ammonia almost all HIn is converted to In^- and a further increase of NH_3 is not matched by the corresponding increase of In^-.

FIA principles for determination of ammonia

Method A (the indophenol method):

Manifold

The sample, S, is injected into a carrier stream, C, which is merged with a solution of sodium tartrate and sodium hydroxide, R1. R2, containing phenol, sodium hydroxide and nitroprusside and R3, containing sodium hypochlorite are added and the mixture is heated. The detector wavelength should be in the range 620 - 700 nm, preferably 690 nm for FIA, see Fig. 9.4. Matrix matching might be necessary for certain samples.
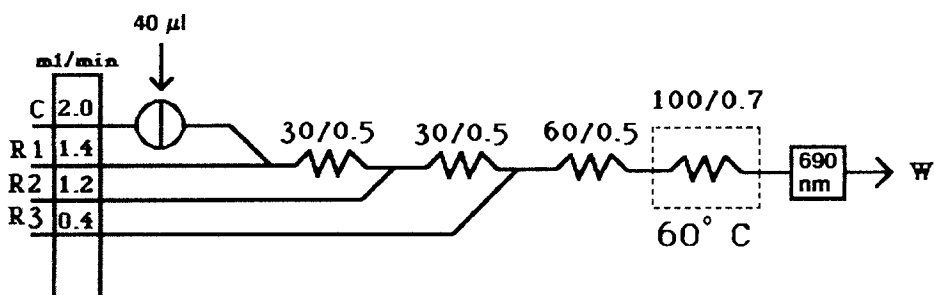

Fig. 9.4. Manifold for determination of ammonia according to the indophenol blue method. Working range: 10 - 100 ppm. Numbers above coils denote coil length/i.d. (cm/mm).

Reagents

C   Distilled water.

R1   15 g sodium tartrate and 20 g sodium hydroxide are dissolved in 500 ml distilled water.

R2   42 g phenol, 18 g sodium hydroxide and 0.6 g nitroprusside are dissolved in 500 ml distilled water.

R3   10% sodium hypochlorite solution.

Working range

The manifold and the reagents specified will give a working range of about 10 - 100 ppm.

Interferences

Very few interferences other than color of the samples have been reported for this method.

Method C (the gas diffusion method):

Manifold

The sample, S, is injected into a carrier stream, C, which is merged and mixed with sodium hydroxide, R1. This joint stream is passed along the gas permeable membrane. The indicator stream, R2, flows on the other side of the membrane and onto the flow cell where the color change caused by the ammonia gas is detected, see Fig. 9.5.

The carrier stream, C, consists of distilled water (free of carbon dioxide), i.e. matrix matching is not normally needed.
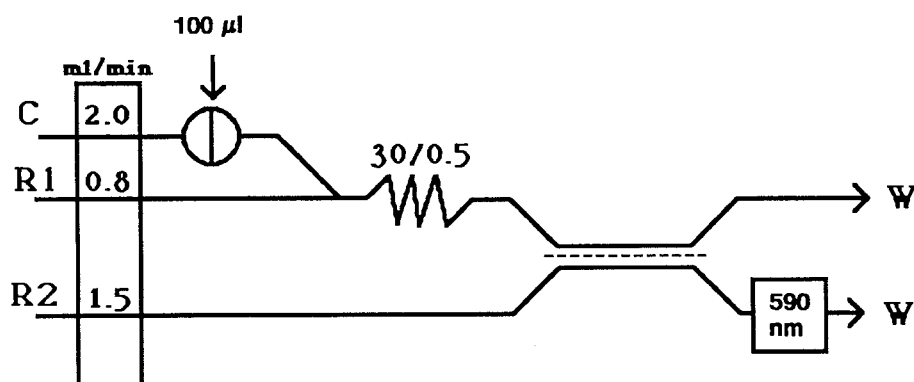

Fig. 9.5. Manifold for determination of ammonia according to the gas diffusion method. *Working range:* 1 - 10 ppm. Numbers above coil denote coil length/i.d. (cm/mm).

Reagents

R1   0.1 M sodium hydroxide.

R2   Indicator solution (Tecator indicator mixture, 0.1 g/l) adjusted with small volumes of either sodium hydroxide or hydrochloric acid to obtain an absorbance value of 0.25 - 0.30 A.U. at 590 nm which occurs at a pH value of about 6.4. Note that this solution is extremely sensitive to impurities of acids and bases which might be present on the walls of the glassware used. The absorbance and pH of R2 may change with time due to slow uptake of carbon dioxide or due to dissolution of impurities from the storage vessel. Usually a stock solution of the indicator mixture is prepared (5 g/l). This stock solution is stable for several months. The working indicator solution, R2, must be prepared daily. Filter and degas before use.

Working range

For maximum sensitivity use a carefully prepared R2 solution with a correct pH value of 6.4. The buffer capacity should be as small as possible (impurities will increase the buffer capacity). The injection volume should be at least 200 μl. If a linear gas diffusion cell with groove lengths on the order of 7 cm is used along with flow rates according to Fig. 9, a working range of 50 - 1000 ppb ammonia (with respect to N) is readily obtained. By increasing the groove lengths, e.g. by using a gas permeable membrane in a dialysis unit such as the Tecator 5106 Module (with groove lengths of 50 cm), a factor of 3 - 5 improvement in sensitivity is accomplished. For higher working ranges the indicator solution R2 must be buffered. Prepare a 0.1 M sodium dihydrogen phosphate stock solution. Add about 1 ml of this stock solution per liter of R2 to reach a range of 1 - 10 ppm ammonia (with respect to N), 5 ml to reach 10 - 100 ppm and 30 ml to reach 100 - 1000 ppm. In all cases the pH of R2 is adjusted to 6.4. Furthermore, the injection volume should be reduced to 30 - 40 μl. For extremely high ammonia concentrations in the sample hydrochloric acid can be added to R2.

Interferences

Volatile amines with low molecular weight will interfere. Very few other interferences can be expected.

<u>Standard stock solution, 1 g N/liter</u>

Dissolve 3.819 g anhydrous ammonium chloride dried at 100 °C in distilled water and dilute to 1 liter. Store in an amber glass bottle at 4 °C. If a drop of chloroform (preserving agent) is added this standard solution is stable for several months.

9.5   RESIDUAL OZONE

The determination of residual ozone has been an extremely difficult method to develop because of the various potential interferences that exist in the sample. Most chemical batch methods are based on iodometric chemistry. Unfortunately, oxidizing agents other than ozone will interfere making the method non-selective. Iodometric chemistry is also very reactive with other oxidizers. This determination is complicated further by the fact that ozone rapidly decomposes at the normal pH values of water. The decomposition products include peroxide, superoxide and hydroxide ion.

The need to accurately determine the residual ozone concentration is directly tied to the efficiency of the water treatment facility. The production of ozone is expensive due to the energy needed for generation. The closer the facility is monitored for complete disinfection, the less excess ozone will be produced and the energy consumption is thereby optimized.

The best batch method for the determination of ozone is the indigo blue method (13,14). Its principle is that the ozone attacks the double bond of the blueish purple indigo dye cleaving it into to noncolored species. The degree of decoloration is proportional to the original ozone concentration. Chlorine and manganese ions are potential interferences.

The biggest difficulty with the batch method is to reproducibly handle the ozone sample. The high volatility of the analyte along with the decomposition means that the potential for random results exists. Furthermore, the modifications needed to eliminate the interferences add more handling steps to the procedure thereby complicating the precision problem.

The interference problems can be eliminated by incorporating a gas diffusion manifold in an FIA system (15,16). The gas diffusion system separates the ozone from interfering metal ions and peroxide. The membrane also restricts the amount of chlorine which passes into the acceptor stream where the measurement takes place. The rate of the ozone/indigo reaction is faster than the chlorine/indigo reaction thereby producing the kinetic discrimination needed to essentially eliminate the chlorine interference.

FIA principles

Fig. 9.6 shows the FIA manifold. The carrier, C, is continuously pumped by pump 1. The sample, S, is injected into C at time T_1, see Fig. 9.7, and passed along the gas diffusion membrane. The reagent is pumped using a second pump as the acceptor stream. It is stopped at time T_1 for a period of 10 - 30 seconds depending on the sensitivity desired. During the stopped period T_1 to T_2, see Fig. 9.7, the ozone from the continuously passing sample penetrates the membrane in sufficient quantities to allow the measurement to be made. When pump 2 is restarted at time T_2, all sample has left the diffusion area and the donor stream is again the carrier. The absorbance of the acceptor stream is measured at 600 nm.
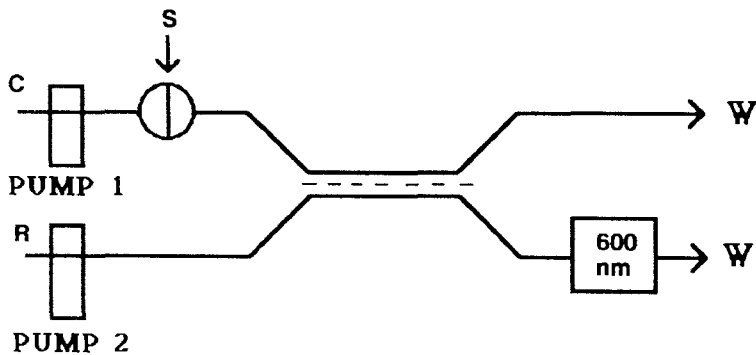

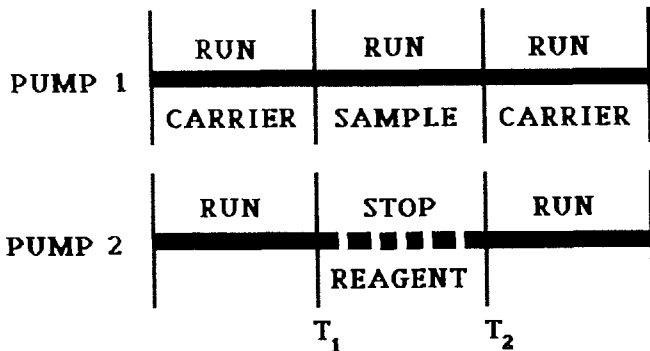Fig. 9.6. Manifold for the determination of ozone using gas diffusion.

Fig. 9.7. The timing sequence for the two pumps used in the ozone determination.

Reagents

R   An indigo reagent stock solution is prepared by adding 770 mg of potassium indigo trisulfonate to a 1 liter volumetric flask containing approximately 500 ml of distilled water, 1 ml of concentrated phosphoric acid and 10 g sodium dihydrogen phosphate. This mixture is then diluted to 1 liter with distilled water. This stock solution is stable for approximately 4 months when stored in the dark. The stock solution should be discarded when the absorbance of a 100-fold dilution falls below 0.16 absorbance units/cm. The concentration of the reagent used in the acceptor stream is either 15 or 77 mg/l depending on the sensitivity needed for the determination. To prepare these reagents transfer 20 and 100 ml of the stock solution respectively into a 1 liter volumetric flask containing 10 g sodium dihydrogenphosphate and 7 ml of concentrated phosphoric acid. Dilute to 1 liter with distilled water. The pH of R is about 2 or less.

C   The carrier is identical with R except that potassium indigo trisulfonate is omitted.

Calibration of the system

Calibration of ozone methods is difficult. The indigo method has been adopted since a simple relationship has been established between ozone concentration (mg/liter) and the absorbance, A:

$$[O_3] = (A * 100) / (f * b * v)$$

where v is the volume (liter), b is the detector cell path length (cm) and f is the calibration factor with a value of 0.42 when based on an ozone molar absorptivity of 2950 M^{-1} cm^{-1} (258 nm). The f value needs to be reevaluated for each dye lot. The purity of the dye varies between lots and manufacturers. Calibration of the dye is accomplished using a UV-visible spectrophotometer at 258 nm.

Discussion

It is clear from table 9.1 that the GDFIA procedure is the best method in terms of interferences. The linear range and sensitivity can be changed by using different concentrations of the indigo solutions or by variation of the stop times. The batch method

is usually run using a 10 cm cell while both FIA procedures use the standard 1 cm flow cell.

TABLE 9.1

Comparison of Batch, FIA and GDFIA Indigo Method for Ozone

PARAMETER	BATCH	FIA	GDFIA
Sampling frequency, samples/hour	20	120	65
Linear range, ppm O_3	0.05-0.5	0.2-4.0	0.03-0.4
Sensitivity, A.U./ppm O_3	0.42	0.046	0.118
Interferences reported, expressed as apparent ozon, ppm:			
H_2O_2	none	none	none
Mn(VII), 1 ppm	0.83	0.44	none
Cl_2, 1 ppm	- -	0.36	0.008

9.6   SULFATE

There are several analytical methods for determination of sulfate in water. All methods involve barium in one way or another (17-19). Barium sulfate has a limited solubility in water which means that the precipitate per se can be detected turbidimetrically. Alternatively, the decoloration of a barium complex due to barium sulfate formation can be measured spectrophotometrically. Removal of cations in the sample prior to addition of Ba^{2+} is in many cases necessary. Conditioning of the flow system is also desirable since otherwise the barium sulfate precipitate may cause coating and clogging. All the mentioned analytical steps can be implemented in the FIA method.

Sample pretreatment

The sample aliquot should either be free from turbidity or be filtered through a 0.45 μm filter.

Chemical principles

(A)   The turbidimetric method.

        The principle is turbidimetric determination of sulfate as barium sulfate.

(B)   The MTB method.

        Methylthymol blue (MTB) forms a blue complex with barium ions in alkaline solutions. When sulfate is added barium sulfate is formed and the complex concentration decreases. This decrease is measured at 620 nm. Alternatively, the increase in uncomplexed methylthymol blue concentration can be measured at 470 nm (less sensitive than the 620 nm method).

FIA principles for sulfate determination

Method A (turbidimetric method):

Manifold

        The sample is injected into an acidic carrier stream which is merged with a barium chloride stream and mixed in a long coil, see Fig. 9.8. The turbidity is measured at 410 nm. At about 30 sec after sample injection the second pump is programmed to deliver EDTA solution during 20 sec to condition the flow system. Solid $BaSO_4$ will then dissolve due to formation of the barium-EDTA complex.
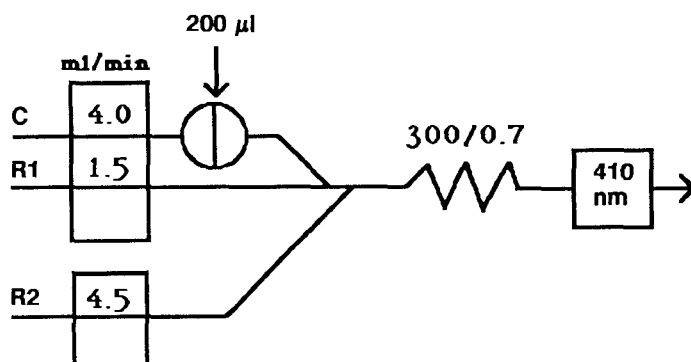

Fig. 9.8. Manifold for turbidimetric determination of sulfate, 100 - 1000 mg/l. Numbers above coil denote coil length/i.d. (cm/mm).

Reagents

C   Distilled water acidified with 4 ml 1 M HCl per liter.

R1   5% (w/v) barium chloride dihydrate in distilled water provided with polyvinylalcohol (0.05% w/v).

R2   0.3% (w/v) EDTA (disodium salt) in 0.2 M NaOH.

Method B (spectrophotometric method):

Manifold 1

The sample is injected in an aqueous carrier, passed through a cationic exchange column, merged with a barium-methylthymol blue solution and finally merged with an alkaline water/ethanol solution. The absorbance decrease is measured at 620 nm, see Fig. 9.9.
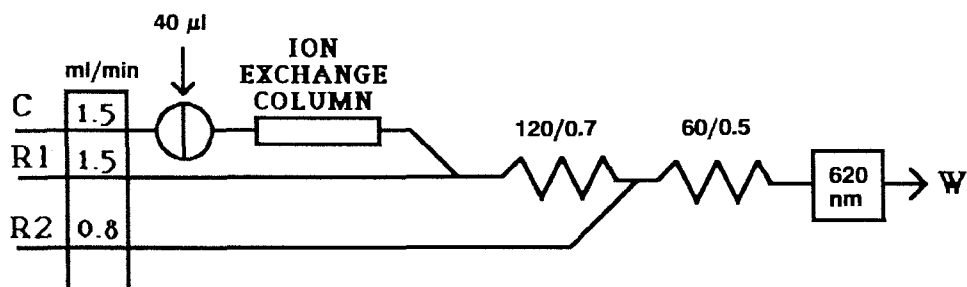
Fig. 9.9. Manifold for determination of sulfate according to the MTB method, 5 - 30 mg/l. When the injection volume is 200 μl the working range is 1 - 10 mg/l. Numbers above coils denote coil length/i.d. (cm/mm).

Reagents

C   Distilled water.

R1   To prepare 1 liter of this solution, use 0.116 g methylthymol blue (sodium salt), 6.0 ml 1 M HCl, 80 ml distilled water and 14.0 ml $BaCl_2$ solution (1.526 g $BaCl_2*H_2O$ per liter). Make up to volume with 95% ethanol. Use a yellow pump tube (solvent flexible) to pump R1.

R2   0.036 M NaOH in 44% ethanol. Use a yellow pump tube to pump R2.

Column preparation

Fill a PVC tube (i.d. 2 mm, length 5 cm) with the H^+ form of a cationic ion exchanger, e.g. Dowex 50 x 8 or Merck Ion Exchanger I. Seal with glass wool or cigarette filter.

Manifold 2

The ion exchanger column is implemented in a prevalve arrangement, see Fig. 9.10. Pump 2 is stopped during the change from one sample to the next sample in order to prevent introduction of air into the column.
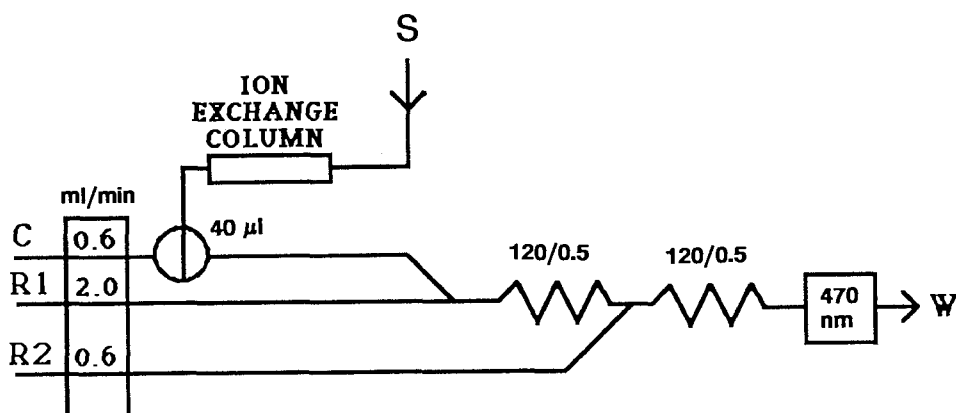

Fig. 9.10. Manifold for determination of sulfate according to the MTB method with prevalve sample treatment, 10 - 125 mg/l. Numbers above coils denote coil length/i.d. (cm/mm).

Reagents

C   Distilled water.

R1   To prepare about 600 ml of this solution, use 0.1182 g methylthymol blue (sodium
        salt) dissolved in 25 ml $BaCl_2$ solution (1.526 g $BaCl_2$ x 2 H_2O per liter), 4.0 ml 1 M
        HCl, 320 ml distilled water and 250 ml 99.5% ethanol. Degas carefully. Use a yellow
        pump tube (solvent flexible) to pump R1.

R2   0.04 M NaOH.

Column preparation

        The column preparation is the same as described in the previous section.

Working range

        Typical working ranges are: 100 - 1000 mg SO_4/l for method A (Fig. 9.8), 1 - 30 mg
SO_4/l for method B, manifold 1 (Fig. 9.9), and 10 - 125 mg SO_4/l for method B, manifold
2 (Fig. 9.10).

Interferences

        Sulfide, sulfite and chromate ions interfere strongly.   Cations (other than H^+)
interfere for the methylthymol blue methods but should not be present after the sample
treatment in the ion exchange column. Chloride ions of very high concentrations (above
1000 mg/l) might interfere in the turbidimetric method. Levels of interference for other
anions: fluoride - 2 mg/l, nitrate - 50 mg/l and phosphate - 2 mg/l.

Standard stock solution, 1000 mg/l SO_4

Dissolve 1.479 g anhydrous sodium sulfate in distilled water and dilute to 1 liter. Store in
an amber glass bottle.

9.7 ALKALINITY

Alkalinity is a measure of the buffering capacity for water or, more specifically, the quantitative capacity to react with hydrogen ions. It is sometimes called total alkalinity or methyl orange alkalinity. A common method for determination of alkalinity is potentiometric titration with hydrochloric or sulfuric acid either to a pH value of 5.4, while continuously purging the sample with carbon dioxide-free air, or to a pH value of 4.5 in a first step and then further to reach a final pH value of 4.2 (20-23). If X_1 equals ml of acid needed to titrate to pH 4.5 and X_2 is the total volume of acid, then the alkalinity is calculated using the quantity $(2X_1 - X_2)$ ml. Alkalinity is expressed in mmol HCO_3^-/l or in mg HCO_3^-/l (1 mmol HCO_3^-/l = 61 mg HCO_3^-/l).

Sample pretreatment

The sample container should be tightly capped as soon as the sample has been collected and the alkalinity determined as soon as possible after the container is opened, preferably the same day. Let the sample settle prior to analysis. The sample aliquot should be free from turbidity.

FIA principle for determination of alkalinity

Manifold

The sample, S, is injected into a carrier stream, C, and merged first with a phthalate buffer solution, R1, and then with a methyl orange indicator solution, R2, see Fig. 9.11.
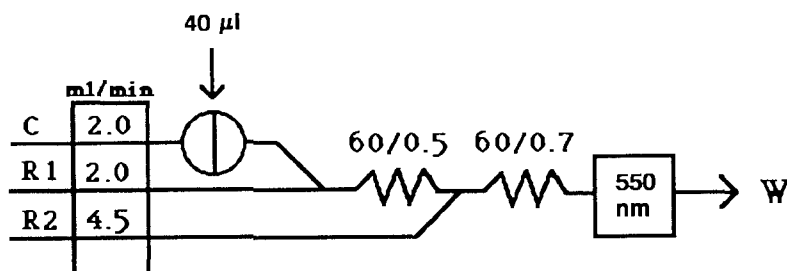

Fig. 9.11. Manifold for determination of alkalinity. Numbers above coils denote coil length/i.d. (cm/mm).

Reagents

C   Distilled water (carbon dioxide-free).

R1   Dissolve 10.2 g potassium hydrogen phthalate in about 500 ml distilled water, add
        175 ml 0.1 M HCl, make up to 1 liter with distilled water. Use a tenfold dilution of
        this buffer as R1.

R2   Make a methyl orange stock solution, 0.05% w/v in distilled water. Use a fivefold
        dilution as R2.

Standard stock solution

        Sodium carbonate standard, 5.00 mmol/l: Dry anhydrous sodium carbonate
overnight at 105 °C. Cool in a desiccator, then weigh 0.530 g and transfer to a 1 liter
volumetric flask. Add distilled water and dissolve. Make up to volume with distilled water.

9.8 FLUORIDE

        Determination of fluoride in water samples with a fluoride selective electrode has
become an officially accepted method due to the fact that this electrode is one of the best
behaved ion selective electrode, ISE (24). When this electrode is operated in batch or
segmented flow mode steady state potential readings are used. Too often the analyst has
to apply an arbitrary interpretation of what should be meant by a steady state potential.
This means that the exposure time may vary from sample to sample. Under flow injection
conditions the exposure time is exactly defined and the potential readings are not
necessarily performed at steady state conditions. An interesting question then is whether
the electrode still obeys Nernst's law or, at least, if it produces a useful calibration graph.
In this respect, the difference between the batch approach and the FIA approach is small.
However, all concerns about electrode individualilty and matrix variations are equally valid
for both approaches. In the case of fluoride determination this means pH and ionic
strength adjustment as well as addition of metal chelating compounds to release fluoride
from its metal complexes.

FIA principle for fluoride determination (25-27)

The sample, S, is injected into a carrier stream, C, and merged with a total ionic strength adjustment buffer (TISAB), R, see Fig. 9.12.

To prevent "overflow" liquid is pumped out from the electrode flow cell at a much higher rate than all incoming flows together. The liquid level in the cell is thereby kept constant.
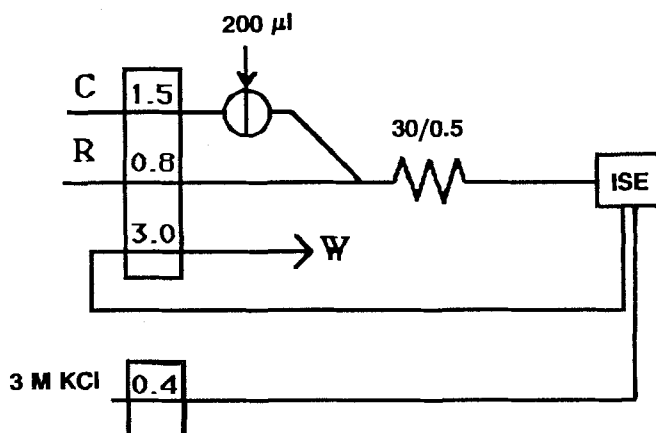

Fig. 9.12. Manifold for fluoride determination with a fluoride selective electrode (ISE). Numbers above coil denote coil length/i.d. (cm/mm).

Reagents

C   A carrier stream containing small amounts of fluoride is recommended to avoid electrode drift, e.g. 20 μg F/l.

R   0.25 M hexamethylentetraamine (HMTA) and 1 M KCl. This solution is prepared as follows: 17.5 g HMTA and 37.8 g KCl are dissolved in 500 ml distilled water. pH is adjusted to 5.5 using concentrated HCl.

Reference electrode

A calomel electrode is used as a reference electrode. To avoid poisoning and malfunction of this electrode a strong KCl solution, 3 M, can be pumped intermittently to renew the liquid in the vicinity of the diaphragm.

Working range

A typical working range is 0.1 - 2 mg F/l. Detection limit should be at least 30 μg F/l with an appropriate flow cell design.

Standard stock solution, 100 mg F/l

Dissolve 0.305 mg potassium fluoride in 1000 ml distilled water.

Interferences

Hydroxide ions interfere but by keeping the pH at 5.5 this interference can be minimized. Ions like Al^{3+} and Fe^{2+} will form complexes with fluoride at a pH below 5. Acetate ions might interfere.

General comments on measurements with ion selective electrodes

Response time measurements of ion selective electrodes have been studied extensively and are beyond the scope of this book. However, response time changes which are associated with the sample matrix are of importance in FIA potentiometry since they directly affect the accuracy and overall reliability of the method. It must be remembered that each electrode might exhibit individual changes in response characteristics with time due to aging and changes in the surface properties. Therefore, there will not be any consistency among different electrodes.

Sample viscosity could have two roles to play in the FIA potentiometric system. First, the higher hydrodynamic resistance after injection of viscous fluids decreases the flow rates and, therefore, affects dispersion. Second, the thickness of the electrode boundary layer at the sensing membrane affects the resonse time. However, upon investigation the sample viscosity produced by a 10% glycerine solution is minimal. As a rule, the viscosity exhibited by this solution is considerably higher than would be expected for samples.

As the ionic strength increases the response time increases for the fluoride electrode. At 50% of the steady state signal the response times were 3.0, 3.4, 3.9 and 5.9 seconds for 0.0, 0.2, 0.5 and 1.0 M sodium chloride, respectively. A second concern is the short transient in the positive potential direction at the beginning of the signal. The fluoride sample creates a positive transient because the solution of higher ionic strength enters the cell. Then, after the sample is removed, a negative transient occurs because the lower ionic strength solution enters the cell. Eventually, the previously established baseline is reached.

The pH dependency of the fluoride electrode is well documented. At lower pH the hydrogen fluoride complexes form. This produces an apparent lower fluoride activity. In the case with FIA a pH between 4.4 - 5.6 is required.

9.9 HEAVY METALS

Heavy metals in water samples are often determined by flame atomic absorption spectrometry. This technique is less sensitive than the graphite furnace technique which, on the other hand, is more expensive and more subjected to interferences. Several reports on preconcentration of heavy metals using FIA have been published using atomic spectroscopy. The simplest method is a single-line FIA system with a column containing a cation exchanger. The sample is injected in a first step whereby metal ions with positive charge are trapped in the column. In a second step the metal ions are eluted by injection of a strong acid. At the same time the column is reconditioned and is then ready for the next sample. The main drawback with this approach is that sample matrix material will pass through the detector during the preconcnetration step. Therefore, the method described here is based on the principle that the two steps, preconcentration and eluation, are performed in two different but integrated flow systems. Furthermore, by operating the column in a countercurrent mode during the eluation step the trapped cations are transplanted into a very small segment of new matrix and do not spread out axially as they would if they were passed through the column.

Sample pretreatment

Water samples are preserved at collection by addition of concentrated nitric acid to a final concentration of about 1 mM of the acid. Prior to analysis 20 ml of each sample is transferred to a 25 ml volumetric flask, neutralized with (1+1) ammonia liquor to the yellow color of methyl orange indicator (2 drops added), buffered with 2 ml of 1 M ammonium acetate at pH 5.5 and diluted tovolume with distilled water. Corrections in the final result are made to compensate for the sample dilution (28-30). Alternative pretreatment procedures should be considered for specific samples and applications.

Principles for the determination of heavy metals

Manifold 1

The principle is shown in Fig. 9.13. Pump 1 is operating, pump 2 is stopped. The sample, S, is injected into a carrier, C1, and passed through an ion exchange column, I.E.C., and further to waste. Detector carrier solution, C2, is pumped in a separate line using the same pump, pump 1. When all sample material (except the trapped cations) has left the column, pump 1 is stopped and pump 2 is started. Eluant, R1, is pumped countercurrently through the column and directly to the detector. After detection pump 1 is started again and pump 2 is stopped.
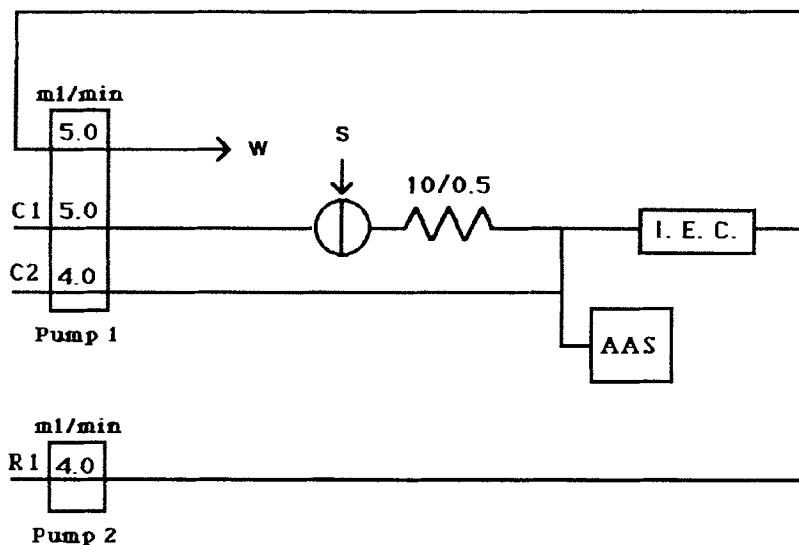
Fig. 9.13. Manifold for determination of heavy metals using a flame AAS detector. For details, see text. Numbers above coil denote coil length/i.d. (cm/mm).

Manifold 2

This is an alternative approach to the two-pump system described above. It requires a programmable two channel injector. Figs. 9.14 a and b show the flow principle. The sample, S, is injected into a carrier, C, and passed through an ion exchange column, I.E.C., which is located in the loop of the second injector channel. This is the

preconcentration cycle (Fig. 9.14 a). The second injector channel is identical with the first channel from a functional point of view but has, for clarity, been drawn differently showing the position of the column. During the eluation step, see Fig. 9.14 b, the column is "injected" into the R stream which serves both as eluant and detector carrier.
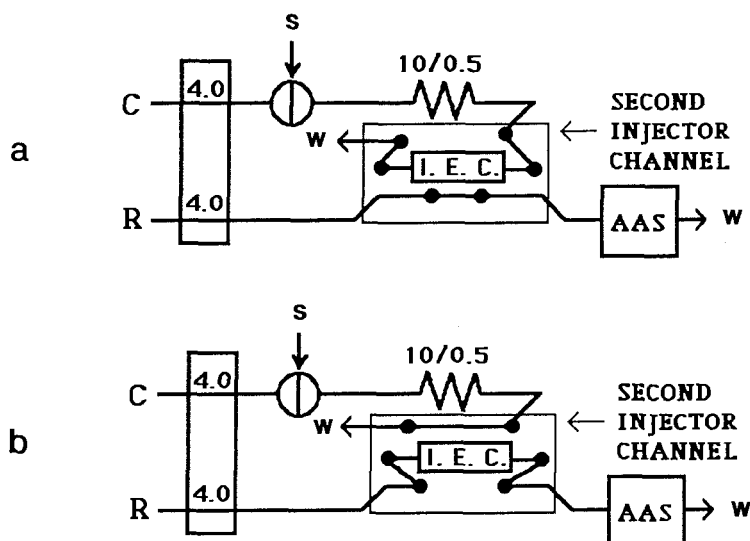

Fig. 9.14. Manifold for determination of heavy metals using a flame AAS detector. (a) preconcentration cycle, (b) eluation cycle. For details, see text. Numbers above coil denote coil length/i.d.

The acid eluant/carrier stream, R, is introduced continuously into the detector. This is in contrast to the previously described approach in which the eluant is introduced intermittently.

Reagents

C   0.1 M ammonium acetate solution adjusted to pH 5.5.

R   2 M nitric acid.

Column preparation

Suitable column dimensions are 40 mm in length and 2.5 - 3.0 mm in inner diameter. A section of a pump tubing can be used. One end of the tubing is sealed with a nylon screen, 100 mesh.

(a) Chelex-100

This ion exchanger is available from Bio-Rad Laboratories, USA, in its sodium form as 50 - 100 mesh. The ion exchanger is converted to its H^+-form before it is filled into the column. The exchanger can be filled as a slurry using a plastic syringe. Since the Chelex-100 resin swells when converted from its H^+-form to its NH_4^+-form special care must be taken upon packing. About 75% of the total column volume is filled with the H^+-form of the exchanger. The other end of the column is then sealed as described above. The chelating cycle, i.e. the time during which the ammonium acetate carrier is transporting the sample through the detector, should not exceed 90 sec. The ion exchange column should be rinsed with water and filled with water when not in use.

(b) 8-quinolinol

This ion exchanger is available from Pierce Chemical Co., USA. It is azo-immobilized to controlled pure glass (CPG, 50 nm pure size and 125 - 177 μm particle size). The ionic form is not important since it does not exhibit the kind of swelling properties as the Chelex-100 does. The total volume of the column can thus be filled with this exchanger.

Standard stock solution

Aqueous standard solutions for most heavy metal ions are commercially available. The usual concentration level is 1000 μg/ml.

Programming of pumps and injector

Manifold 1

Sample volume (injection loop volume): 3 ml
Pumping rate for sample (into the injector): 6 ml/min
Sample aspiration time: 60 sec
Sample injection time: 60 sec
Sampling frequency: 30/h

Manifold 2

Sample volume (injection loop volume): 2 ml
Pumping rate for sample (into the injector): 4 ml

Sample aspiration time: 60 sec

Sample injection time: 60 sec

Sampling frequency: 30/h

Pumping of sample into the injector is preferred to aspiration due to the relatively high flow rates recommended. A large variation of sample volume and manifold flow rate parameters is possible. For large preconcentration factors the sample volumes used should be increased.

9.10   CYANIDE

The determination of anions has always been complicated by the fact that there are few anionic reagents available and the fact that, with the exception of ion selective electrodes, most detectors do not exhibit selectivity for anions. A noteable family of detectors, atomic spectroscopy, cannot be used to directly determine anions. The use of atomic spectroscopy is desirable because of its high selectivity and sensitivity.

The reason that atomic spectroscopy has not been done with anions is the fact that most nonmetals have their resonance lines in the vacuum ultraviolet region. This places an enormous constraint on the instrumentation. To make anionic determinations routine is not trivial both in cost and training. The alternative is to indirectly determine the anion. Utilizing some type of metal anion chemistry, the anion of interest is converted or replaced in the sample zone by a metal. In most cases this implies that some type of reactor must be used to facilitate this conversion.   Under manual conditions the reproducible manipulation of the sample with the reactor is not very good. If this process could be automated then the utility of this type of procedure would be greatly enhanced.

In the case of cyanide such a method was reported using FIA (31). This method uses an on-line heterogeneous chemical reaction utilizing a column containing copper(II) sulfide. Copper(II) is known to be a powerful oxidizing reagent in the presence of cyanide, due to the formation of extremely stable cuprocyanide complexes. The cyanide in the sample readily dissolves the copper sulfide in the reactor. The released copper complexes are determined directly using atomic absorption.

Reagents

The carrier stream is composed of potassium hydroxide at pH 11.0. Potassium cyanide is dissolved in water and standardized using silver nitrate. A stock solution of 0.102 M is prepared. The pH of all standard cyanide solutions are adjusted to 11 using potassium hydroxide.

Microcolumn construction

A reactor body of a chromatographic column consisting of a 6 cm length of borosilicate glass can be used. The ends are equipped with standard luer fittings. A polyethylene porous bed support is fixed in the column tips. The volume of the column is reduced by inserting a thick walled silicone tube inside the column. The final diameter should be about 1 mm i.d. The packed column should contain 150 mg of crushed cupric sulfate particles which are about 0.2 - 1 mm. The cupric sulfide is packed into the column by using a disposal pipet with a tip opening of about 1 mm. The pipet not only acts as a funnel but ensures that no particle larger than 1 mm will be placed inside the column. An additional on-line filter, 3 mm thickness made from medium porosity fritted glass disk, is inserted into 5 mm i.d. by 2.3 cm long tygon tubing and mounted on one end. The two ends of the reactor are connected to the system using thick walled silicone tubing with a diameter of 1 mm i.d.

FIA manifold

The FIA manifold is a simple single line system, see Fig. 9.15. The samples are injected into the carrier stream of potassium hydroxide which is flowing at 6.4 ml/min. The sample size is between 50 - 150 μl. The sample peak begins typically about ten seconds after injection and returns to baseline after about 90 seconds. A throughput of forty-five samples per hour can be achieved.
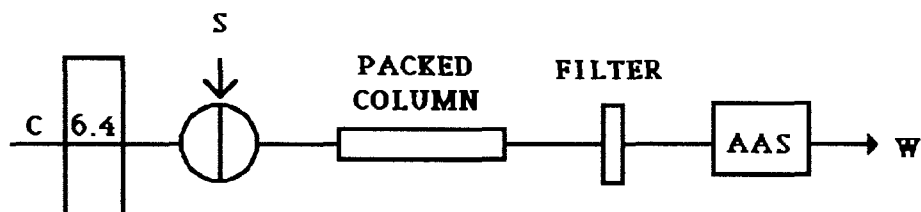

Fig. 9.15. Manifold for the indirect determination of cyanide using FIA.

Results

The conversion efficiency of this reactor is about 33%. A detection limit of 1 ppm may be realized. A typical working range is 1 - 50 ppm. Interference studies at 520 ppm show that most common anions, e.g. acetate and oxalate, exhibit a negative 0.6% interference on a 26 ppm cyanide sample. The only serious interferent appears to be citrate ion. This work demonstrates the advantages of operating a heterogeneous reaction under FIA conditions. It also demonstrates the usefulness of conversion techniques. The selectivity of the heterogeneous reactions make the promise of other conversion systems under FIA conditions bright.

REFERENCES

1.   Standard Methods for the Examination of Water and Wastewater, 14th ed., 1974, part 425E. [-]

2.   Analytical Methods Manual 1979, Inland Waters Directorate, Canada, NAQUADAT Nos 15254 and 15413. [-]

3.   U.S. Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastes, 1971. [-]

4.   American Society for Testing and Materials, ASTM Standards. Part 31. Water, p.448, D 515-72, 1977. Philadelphia, PA. [-]

5.   Analytical Methods Manual 1979, Inland Waters Directorate, Canada, NAQUADAT No 07106. [-]

6.   U.S. Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastes, 1974. [-]

7.   A.W. Morris and J.P. Riley, Anal. Chim. Acta, 29 (1963) 272. [-]

8.   P.G. Brewer and J.P. Riley, Deap-Sea Res., 12 (1965) 765. [-]

9.   J.E. Harwood and D.J. Huyser, Water Res., 4 (1970) 501. [-]

10.   J.E. Harwood and D.J. Huyser, Water Res., 4 (1970) 695. [-]

190

11.   L. Solorzano, Method. Limnol. Oceanogr., 14 (1969) 799. [-]

12.   C. Zadorojny, S. Saxton and R. Finger, J. Wat. Poll. Contr. Fed., 45 (1973) 905.
      [-]

13.   H. Bader and J. Hoigne, J. Water Res., 15 (1981) 449. [-]

14.   H. Bader and J. Hoigne, J. Ozone Sci. Eng., 4 (1982) 169. [-]

15.   M.R. Straka, G. Gordon and G.E. Pacey, Anal. Chem., 56 (1984) 1973. [413]

16.   M.R. Straka, G. Gordon and G.E. Pacey, Anal. Chem., 57 (1985) 1799. [588]

17.   American Public Health Association. Standard Methods for the Examination of
      Water and Wastewater. 14th ed., 47 (1975) 628. [-]

18.   J.M. Adamski and S.P. Villard, Anal. Chem., 47 (1975) 1191. [-]

19.   American Society for Testing and Materials, ASTM Standards. Part 31. Water, 1981.
      Philadelphia, PA. [-]

20.   Analytical Methods Manual 1979, Inland Waters Directorate, Canada, NAQUADAT
      Nos 10101 and 10151. [-]

21.   W. Stumm and J.J. Morgan, Aquatic Chemistry, New York, 1970. [-]

22.   American Society for Testing and Materials, ASTM Standards. Part 23. Water,
      Atmospheric Analysis, p.119,D 1067, 1973. Philadelphia, PA. [-]

23.   U.S. Environmental Protection Agency, Methods for Chemical Analysis of Water and
      Wastes, 1974. [-]

24.   Analytical Methods Manual 1979, Inland Waters Directorate, Canada, NAQUADAT
      Nos 09105 and 09106. [-]

25.   W. Frenzel and P. Braetter, Anal. Chim. Acta, 185 (1986) 127. [870]

26.   W. Frenzel and P. Braetter, Anal. Chim. Acta, 187 (1986) 1. [969]

27.   W. Frenzel and P. Braetter, Anal. Chim. Acta, 188 (1986) 151. [962]

28. Z. Fang, J. Ruzicka and E.H. Hansen, Anal. Chim. Acta, 164 (1984) 23. [497]

29. Z. Fang, S. Xu and S. Zhang, Anal. Chim. Acta, 164 (1984) 41. [498]

30. Z. Fang, S. Xu, X. Wang and S. Zhang, Anal. Chim. Acta, 179 (1986) 325. [743]

31. A.T. Haj-Hussein, G.D. Christian and J. Ruzicka, Anal. Chem., 58 (1986) 38. [671]

CHAPTER 10

PHARMACEUTICAL, CLINICAL, BIOTECHNOLOGY

10.1 INTRODUCTION

These three areas are related because the pharmaceutical products are measured in clinical samples and are sometimes produced by biotechnological methods. However, each area has a unique set of problems that must be resolved for every determination.

In pharmaceutical based analysis there are two basic application. First is the determination of metabolites in clinical type samples and second is the necessity of quality control. The majority of this second workload deals with the quality control issue. Quality control is carried out through the entire production process, from raw material through intermediate and finished purified product to the final consumer product form. FIA can be used at each step of the integrated quality control process. An average size pharmaceutical company may handle and use as many as 1000 different types of raw materials. Unfortunately, it is required that several different determinations are made on each raw material. This means that the total number of methods and variations is extremely large in comparison with, for instance, water analysis. There are, however, some analytical parameters which may appear frequently like "heavy metals", "clarity", "chloride", "sulfate", etc. FIA could definitely be considered in this context even if the number of samples is limited. For the intermediates and for the final products the pharmaceutical company has most likely developed its own analytical methods. Whether FIA would be useful in these situations must be left to the analytical chemist in charge of quality control to judge. Even though there presently is no commercially available FIA system for dissolution rate analysis (DRA), the FIA technique would be ideally suited to handle the samples which are collected from the dissolution vessels. Furthermore, the small volumes required for FIA allow a frequent sample acquisition without altering the original volumes in the vessels.

In clinical analysis the situation is different since "accepted methods" must be applied. These determinations are usually carried out in one of three matrices, blood (serum), urine, and spinal fluid. It should be mentioned that these sample matrices are very complicated. Currently, much of the clinical chemistry is performed using batch methods in the form of kits or, if automated, the batch method is still operated as a

kit-based steady-state system.

The question is; what does FIA have to offer clinical chemists besides the usual high throughput, precision and rapid change of methods? The answer can be focused into three areas: kinetic based measurements, matrix modification, and reagent conservation. Because of the complex nature of the sample matrix, many methods have several potential interferents. One way to eliminate or minimize these interferents is to utilize the kinetic rate difference between the analyte of interest and reagent and the interferent and reagent, kinetic discrimination. A second alternative is to transplant the analyte from the sample matrix to an ideal matrix for detection resulting in selectivity enhancement. Incorporation of gas diffusion, dialysis, and extraction modules as well as columns can accomplish the analyte transplantation. The last advantage of FIA is the conservation of reagents. Many biological based determinations are selectively performed utilizing the specificity of enzymes. Unfortunately, enzymes are usually expensive. A secondary concern is maintaining the activity of the enzyme. By using intermittent pumping or other reagent conservation technique enzyme use can be minimized.

One biotechnological use of FIA is for analysis of fermentation products. Essentially, the FIA system is used to monitor the fermentation process in real time. The difficult part of this process is sample acquisition. Reproducible samples are difficult to obtain. The sample matrix which is heterogeneous in nature makes sample selection irreproducible. The fermentation system can be dramatically affected by the sampling procedure, i.e. stopping the fermentation, infection. The stability of the analyte could add an additional problem to the chemist.

## 10.2   DETERMINATION OF CODEINE IN PHARMACEUTICAL PREPARATIONS

Codeine is a common constituent in many pain relieving pharmaceutical preparations. The procedure described here was originally developed for the determination of codeine in acetylsalicylic acid tablets (1) but can, of course, be adapted to other preparations. The procedure is based on liquid-liquid extraction of the codeine-picrate ion pair into chloroform.

Sample preparation

The tablets are homogenized and an amount corresponding to a final concentration of 3.5×10^{-4} M codeine is dissolved in 0.065 M phosphate buffer, pH 6.5. Filter, if necessary.

Chemical principle

Codeine is extracted in its cationic form, HB^+, according to

$$HB^+_{aq} + P^-_{aq} \rightleftharpoons HBP_{org}$$
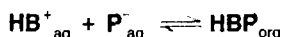
where P^-_{aq} is the picrate anion in the aqueous phase and HBP_{org} is the extracted ion pair. The extraction yield is influenced by the pH of the aqueous sample aliquot. The useful pH range for quantitative formation of HB^+, the codeine cation, is 3 - 7. At low pH values picric acid can be co-extracted and this fact limits the useful pH range further, approximately to 4 - 7. Furthermore, the situation is complicated by the presence of acetylsalisylic acid. At pH values above 6 the presence of undissociated acid in the organic phase is negligible. Thus, the recommended pH is 6.5. The codeine picrate ion pair is measured spectrophotometrically at 405 nm.

FIA principle for codeine determination

Manifold

The sample, S, is injected into a phosphate buffer and merged with a picrate reagent, R1. Water, R2, is pumped into the displacement bottle containing chloroform, Org. The aqueous and the organic streams merge at the segmentation point marked "segmentor" in Fig. 10.1. The extraction takes place in a PTFE coil, length 200 cm, i.d. 0.5 mm. The two phases are separated using a membrane separator and the organic phase is led to the flow cell in the spectrophotometer. The useful wavelength range for measurement of the absorbance is 350 - 410 nm. The manifold design is shown in Fig. 10.1.

Carrier and reagents

C   0.065 M phosphate buffer, pH 6.5.

R1   0.025 M picrate prepared in 0.065 M phosphate buffer (C) adjusted to pH 6.5. Neutralize picric acid with sodium hydroxide in a first step, then add buffer to obtain the final concentration values.
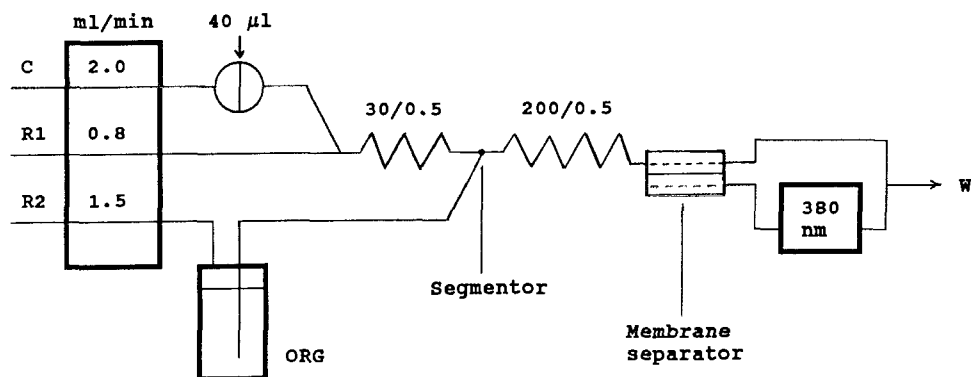
R2   Distilled water.

Org   Chloroform.

Fig. 10.1. Manifold for determination of codeine by liquid-liquid extraction of its picrate ion pair. Numbers above coils denote coil length/i.d. (cm/mm).

Preparation of standard solutions

Use codeine phosphate of pharmacopeia quality. If the tablets contain sodium lauryl sulfate, or any other related substance, the composition of the standard solutions should be adapted to be as close as that of the sample solutions. Influence of the different constituents in the tablet should be investigated by running pure codeine standards spiked with the constituents in question. Prepare standards in the range $2 - 5 \times 10^{-4}$ M.

Concluding remarks

The consumption of organic phase is 1.5 ml/min. Thus, with a sampling frequency of 60 samples/hour only 1.5 ml of organic phase (chloroform) is needed per sample. Manual extraction procedures require larger volumes of both organic phase and sample. The repeatibility of the described method should be better than 1.5% r.s.d. at the defined analyte concentration level, 3.5×10^{-4} M.

10.3   DETERMINATION OF LOWRY PROTEIN

The methods for the determination of protein levels are based on intrinsic properties, chemical reactions, or binding affinities. The most often used method for the determination of protein is the Lowry method. An adaptation of this method to FIA has been described for bovine serum albumin and hepatitis B antigen (2).

FIA principle for determination of Lowry protein

The sample is pumped from the sampler into the injector and injected into a carrier stream, C, comprising either of distilled water or of a phosphate-buffered salt solution (phosphate buffer at pH 7.2 containing 7 mg NaCl, 1.7 mg $NaH_2PO_4 \cdot H_2O$ and 0.2 mg $Na_2HPO_4 \cdot 7H_2O$ per ml). Alkaline tartrate copper reagent, R1, is added and mixed with the carrier (sample) stream and heated in a 100 cm long coil, see Fig. 10.2. Folin-Ciocalteau reagent, R2, is then added and detection is made at 660 nm. A restrictor coil after the flow cell prevents formation of gas bubbles. The wash solution for the sample probe is 0.01 M NaOH.
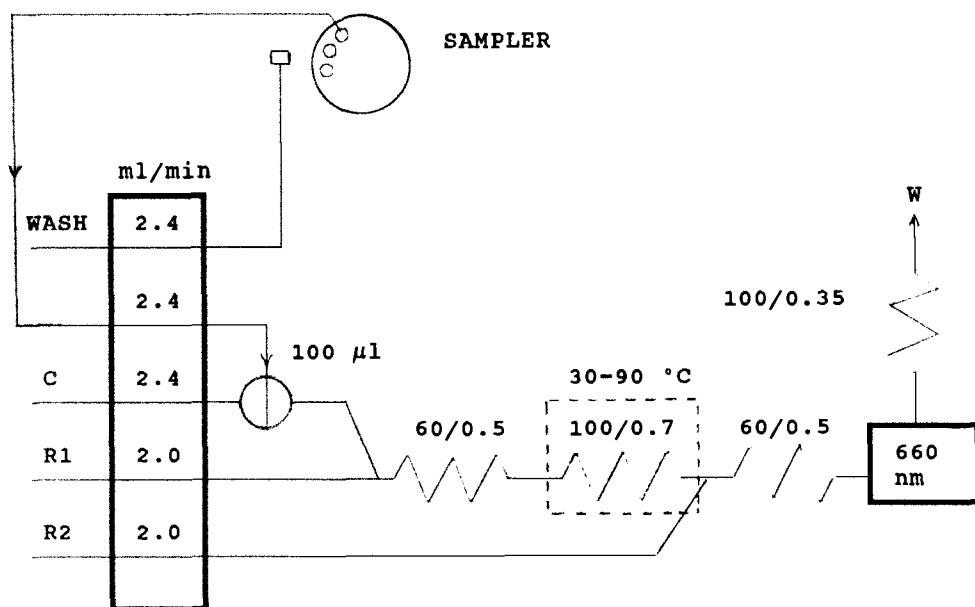
Fig. 10.2.   Manifold for the determination of proteins. Numbers above coils denote coil length/i.d. (cm/mm). For details, see text.

Reagents

All reagents are degassed before use.

C   Distilled water (or buffer, see above).

R1   Mix 100 ml copper sulfate solution, 0.7 mg $CuSO_4 \cdot 5H_2O$ per ml, with 250 ml of alkaline tartrate solution, 18 mg NaOH, 20 mg Na_2CO_3, 1.4 mg $NaKC_4H_4O_6 \cdot 4H_2O$ per ml.

R2   Mix 50 ml Folin-Ciocalteau reagent (as purchased) with 250 ml distilled water.

Results

Temperature directly affects the rate of reaction between the proteins and the alkaline tartrate copper reagent, R1 (2). Therefore, 100 cm of the 160 cm coil used to mix R1 with the sample is placed in a thermostated cell. The increase in signal between 30 and 90 °C is about 70% for the bovine serum albumin and 300% for the hepatitis B surface antigen. At 90 °C both proteins exhibit the same absorbance reading for the same concentration. The response of the method is 20 seconds with a less than 2% r.s.d.

10.4   DETERMINATION OF CREATININE

The determination of creatinine in biological samples is widely used in clinical diagnostics to evaluate renal, thyroid, and muscle function. The creatinine clearance test is an important determination for renal efficiency particularly during artificial dialysis treatments. The usual analytical method is the Jaffe reaction in which creatinine reacts with picric acid to form a colored species which absorbs at 500 nm (3,4). This reaction is not specific and the analytical procedure is time consuming. To achieve the desired specificity enzymatic methods based on creatinase or creatinine iminohydrolase have been developed (5). The problem with these methods is that they involve many expensive enzymes in a series of intricate reaction sequences and are, therefore, time consuming and tedious. Ammonia is the usually monitored end product of these reactions. The ammonia originally present in the sample must be removed or quantitated (blank correction) for each sample. It is clear that FIA can offer several advantages to this method. First, the enzymes can be immobilized in packed bed reactors, thus reducing the cost per sample. The ammonia originally present in the sample can be enzymatically

removed before "new" ammonia is produced by the influence of creatine iminohydrolase. This enzyme reaction would also be performed inside a packed bed reactor. A specific detector such as an ion selective electrode or other electrochemical device can also be incorporated into the manifold. The flowing stream would make it possible to continually clean the surface of the electrode thereby maintaining the desired performance levels.

Yet another method has been worked out which uses enzymes. The enzymes are immobilized on an iridium metal oxide semiconductor, MOS (6). The electrode surface is protected from the solution by a gas permeable membrane. The usual principles of gas diffusion apply for this detector. Only gaseous constituents in the sample diffuse through the membrane to interact with the electrode surface. The reaction chemistry uses two packed bed reactors. The first contains glutamate dehydrogenase. The ammonia in the sample reacts with α-ketoglutamate in the presence of NADH to form glutamate and NAD^+. The creatinine in the sample is then converted to N-methylhydantoin and ammonia using creatinine iminohydrolase, immobilized in the second reactor. A detailed description of this method follows.

<u>Immobilization procedure (6)</u>

The glutamate dehydrogenase (GLDH) is covalently attached to glutardialdehyde activated controlled pore glass (CPG) beads. To a suspension of 0.5 ml of activated CPG in 5 ml of immobilization buffer, 4 mg of glutamate dehydrogenase with a corresponding activity of 144 units is added. The mixture is gently shaken at 4 °C for 12 hours. The CPG is washed twice with 0.5 M sodium chloride and finally, once with the immobilization buffer. The apparent activity on the CPG should be about 70 units/ml.

For the immobilization of creatinine iminohydrolase (CIH) 5 units are added to 0.3 ml of oxirane acrylic beads which are suspended in 5 ml of immobilization buffer. The suspension is gently shaken for 48 hours and then washed twice with water. Then the mixture is shaken with 5 ml of 3 mM glycine in immobilization buffer. This reaction terminates all the unreacted groups on the beads. The beads are washed with 0.5 M sodium chloride twice and immobilization buffer twice. The preparation should carry an apparent activity of 10 units/ml suspended matrix.

The immobilization buffer is 0.05 M sodium phosphate at pH 7.0. The working buffer for the method is 0.05 M Tris-HCl at pH 8.5. This buffer also contains 1.5 mM NADH, 0.5 mM α-ketoglutarate and 3 mM sodium azide as a preservative. The washing buffer is 0.1 M sodium phosphate at pH 7.0 with 0.8 M sodium chloride.

<u>Stock solution</u>

A stock solution of 20 mM creatinine is prepared every three days by dissolving 0.2264 g creatinine in 100 ml of water. When not in use all solutions are stored at 4 °C.

<u>Manifold</u>

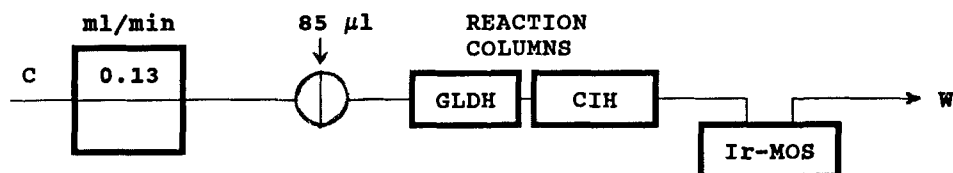The FIA manifold used in the determination of creatinine is shown in Fig. 10.3.

Fig. 10.3. Manifold for the determination of creatinine using immobilized enzymes. GLDH = glutamate dehydrogenase, CIH = creatinine iminohydrolase. For details, see text.

The sample is injected before the reactors. The reactors are packed in PTFE tubing. The glutamate dehydrogenase is in a column, 2 mm i.d. and 30 mm long, containing 94 μl of enzyme preparation with an apparent activity of 6.6 units. The creatinine iminohydrolase is packed in a column, 2 mm i.d. and 40 mm long, containing 125 μl of enzyme preparation with an apparent activity of 1.25 units at pH 8.5. In both cases the enzyme activity is high enough to ensure 100% substrate conversion. Once through the reactors the sample zone passes through the Ir-MOS detector. After the detector a thin 0.2 mm i.d. 0.5 mm long section of PTFE tubing is added to increase the pressure difference across the gas permeable membrane.

Results

The optimum pH for the reactors is from 7.5 to 7.9. However, as the system becomes more basic ammonia formation is favored. At pH 8.5 the creatinine iminohydrolase does not show any loss of activity. While the glutamate dehydrogenase looses about 10% of its activity. Therefore, the system is run at pH 8.5 to maximize ammonia production. The blood and serum samples tested are diluted 25 and 1000 times, respectively. Recovery of the creatinine spiked samples should be in the range of 93 - 105%. Interference levels have been tested for many of the common drugs. With the exception of low molecular weight amines no significant interferences were observed. The lifetimes of the enzyme reactors are several months if stored at 4 °C when not in use. The detection range should be linear from 0.2 to 30 μM. At least 15 samples per hour can be determined. A relative standard deviation of 3 - 4% at a mean creatinine concentration of 100 μM can be expected.

The described FIA procedure is a fast and simple method for the determination of creatinine in whole blood, blood plasma and urine. The specificity of the two reactors and the detector make the need for blank correction unnecessary. The substrate is completely converted making the method very sensitive.

10.5   DETERMINATION OF LACTATE

This application was originally developed for lactate in perchloric acid extracts of capillary blood (7) but can be modified to suit other sample types. Determination of lactate is of particular interest during exercise studies since the increase in blood lactate concentration reflects the gradual shift to anaerobic metabolism in working skeletal muscles.

Sample pretreatment

Capillary blood, 20 μl, is obtained by finger prick and deproteinized with 180 μl 0.3 M perchloric acid. The mixture is shaken for 15 - 30 seconds at room temperature and centrifuged during 1 - 2 minutes at 7000 x g. The clear acid supernatant is used to fill the injector in the FIA system.

Chemical principle

The lactate determination is based on the following reactions

$$\text{lactate} + \text{NAD}^+ \xrightleftharpoons[\text{}]{\text{LDH}} \text{pyruvate} + \text{NADH} + \text{H}^+$$

$$\text{pyruvate} + \text{L-glutamate} \xrightleftharpoons[\text{}]{\text{GPT}} \text{L-alanine} + \text{2-oxoglutarate}$$
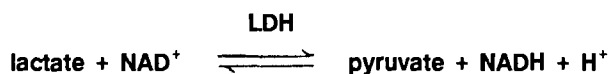
where NAD^+ is nicotinamide-adenine-dinucleotide, LDH lactate dehydrogenase and GPT glutamate pyruvate transaminase. Since the first of these two reaction equilibria is strongly displaced to the left, pyruvate and H^+ must be removed. To this end, the second reaction consumes pyruvate and the alkaline buffer keeps the H^+ concentration at a low level. The increase in NADH concentration is measured fluorimetrically.

FIA principle for lactate determination

Manifold

The manifold used is shown in Fig. 10.4.
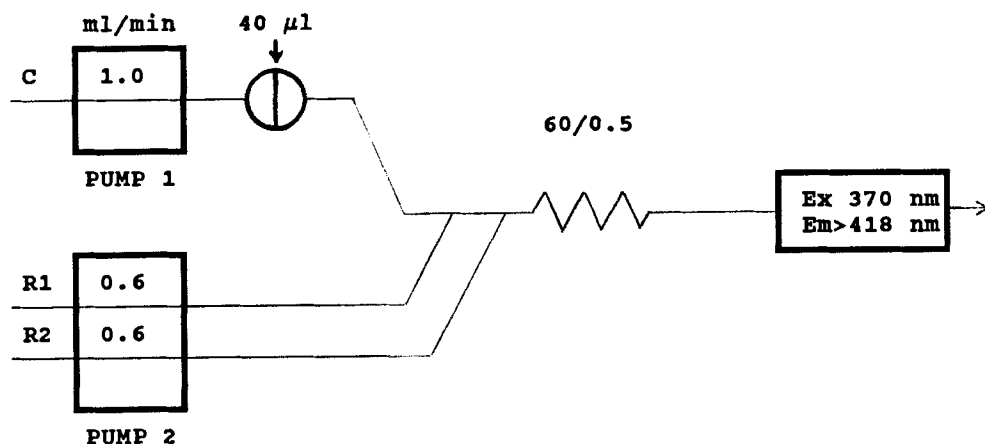
Fig. 10.4. Manifold for determination of lactate. Numbers above coil denote coil length/i.d. (cm/mm).

The sample, S, is injected into an alkaline TRIS buffer carrier, C. Addition of the R1 and R2 reagents is started 1 s after sample injection and stopped 6 s later using pump 2. The entire sample zone is thereby provided with R1 and R2. The fluorescence increase is measured; excitation is performed at 370 nm and emission is measured using a > 418 nm filter in the fluorimeter.

Carrier and reagents

C   Tris hydroxymethyl aminomethane (TRIS buffer), 0.2 M, pH 9.5 ± 0.1.

R1 and R2 are prepared from a Testomar lactate kit (No. OKA, Behringwerke, FRG):

R1   Vial 1, containing the enzymes, is reconstituted with 15 ml of TRIS buffer, pH 9.5. Concentration of the different constituents:
TRIS buffer, pH 9.5, 0.2 M
Glutamate, 22 mM
GTP (glutamate pyruvate transaminase), 2.4 U/ml
LDH (lactate dehydrogenase), 21 U/ml

R2   Vial 2, containing NAD^+ (nicotinamide-adenine-dinucleotide) coenzyme, is reconstituted with 15 ml of water. The final concentration is 3.1 mM.
The reagents are stable for about 7 days at room temperature.

Standard preparation

Prepare standards in the range 0 - 15 mM using lithium lactate having the same perchloric acid concentration as the samples.

Interferences

Pyruvate, initially present in the sample, may suppress the signal. At a lactate concentration of 10 mM a pyruvate concentration of 0.2 mM will give rise to a signal decrease of about 3%. In Table 10.1 the influence of pyruvate concentration is summarized.

<u>Comments on the method</u>

The sampling from a patient of 20 μl capillary blood by finger prick requires skilled personnel. The addition of 180 μl perchloric acid must be performed accurately. The perchloric concentration recommended, 0.3 mM, is the minimum concentration required to obtain a clear, protein-free supernatant in a blood dilution of 1:10. If higher perchloric concentrations are used the detector signal increases.

TABLE 10.1.

Influence of pyruvate, initially present in the sample, on the observed signal. The decrease is expressed as a percentage of the signals obtained for samples containing no pyruvate.

Lactate conc. mM	Pyruvate conc. mM	Signal decrease %
10	0.2	3
2	0.2	9
10	0.5	9
2	0.5	20

The NAD^+ is unstable in alkaline medium. The quality of the NAD^+ is important. Certain brands of NAD^+ may give rise to blank fluorescence. Standards must be run both at the beginning and at the end of a sample batch (maximum 20 samples). Frequent running of a commercial lactate control is also recommended. The calibration graph is non-linear.

The described method uses commercially available and nontoxic reagents. The reagent cost is estimated to be 5 US cents per assay.

10.6   DETERMINATION OF ISOPRENALINE

The determination of isoprenaline is accomplished spectrophotometrically by reacting the isoprenaline with potassium hexacyanoferrate(III) to form the red

N-isopropylnoradrenochrome. In the manual method the reaction is carried out at pH 7.4 and is stabilized 30 seconds later by changing the pH to 4.0. This pH change is necessary to prevent product decomposition. The lack of operator repeatibility in the timing of the addition may lead to losses in precision. Obviously, the incorporation of the method into FIA would eliminate this repeatability problem.

Consequently, a method was developed using FIA (8). But of more significance is the fact that the method was optimized using a four-variable modified simplex optimization procedure. This method is the first which illustrates the use of simplex and it also has a pharmaceutical application. The actual optimization of an FIA method can be difficult to reproduce between laboratories. This is not surprising since in any continuous flow method, in addition to the normal chemical variables of pH, reagent concentrations, temperature, etc., the system parameters of flow rates, tubing length, internal tubing diameters and sample size must also be taken into account. Therefore, in a simple single channel system, there are five separate variables in the system. A solvent extraction manifold can have upwards of 12 variables. The conventional optimization procedure of changing one variable at a time (univariate method) is therefore time-consuming and may be inadequate as a means of reliably determining the best set of experimental conditions.

The modified simplex method for optimization is better suited to this type of system since several variables are changed simultaneously. This has been described as a hill climbing approach to optimization. For example, if there is a four variable system, 5 experiments are required for the first simplex. The four variables can be thought of as four orthogonal axes where each experiment is represented by a point in this factor space. The figure produced by the points of the five experiments is known as a simplex. A four variable system would form a pentagon. After the first set of experiments are concluded, it is easy to determine which experimental conditions produced the best, next best and worst values. By reflecting the worst response value, W, through a point, P, located on the opposite face of the simplex, a reflection, R, is generated, see Fig. 10.5. By rejecting W and replacing it with R a new simplex is formed and by repeating this procedure the simplex climbs the response surface. Several modifications have been made including one which also contracts as well as reflects (8).
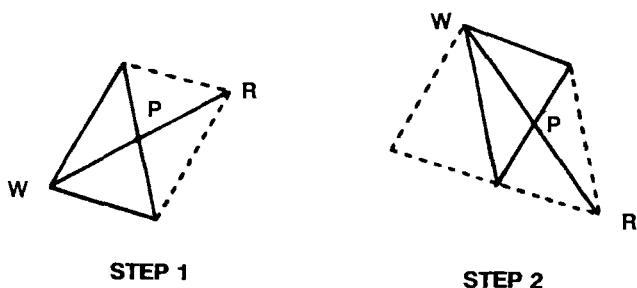

STEP 1   STEP 2

Fig. 10.5. Schematic illustration of the modified simplex method. For details, see text.

The selection of boundary conditions and experimental tolerances is the responsibility of the analyst and care is needed to set realistic values. For the initial simplex, the first points are based on experimental evidence and the others are computer generated. This approach usually covers 10 - 40% of the total range. Therefore, the choice of the first point is to define the area of exploration. For example, sample size can be small and increase instead of large moving to small. The procedure is terminated when an optimum is reached, the analyst feels that the current rate of improvement is not worth the cost or that a predetermined set of experiments have been performed.

Reagents

The potassium hexacyanoferrate(III) stock solution is 0.0243 M. The sodium hydroxide stock solution is 0.200 M. The carrier stream is 20 ml of pH 7.4 buffer. The isoprenaline stock solution, 0.05 g/liter of isoprenaline sulfate dihydrate, should be prepared fresh daily and kept in a dark place.

Manifold

The manifold used is a simple one line system with a spectrophotometer as a detector. The detector used has a 1.4 mm cell path but a conventional flow through detector will work. The manifold used is shown in Fig. 10.6.
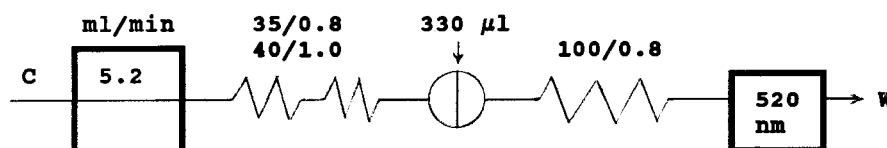
Fig. 10.6. Manifold for the determination of isoprenaline. Numbers above coils denote coil length/i.d. (cm/mm).

Results

According to reference 8 the optimized procedure for the four variable method with high throughput and fixed 330 μl sample loop was 100 cm coil length, 5.2 ml/min flow rate, 7.87 pH, 4.13 mM ferricyanide. The throughput was 150 samples per hour. The sensitivity was 80% of maximum. The FIA procedure developed by the four variable modified simplex optimization takes about 60 seconds per analysis but takes 25 seconds at conditions which produce signals which are 80% of maximum sensitivity. The reproducibility of sample residence time in the flow system is such that a change in pH is not required. The method has been converted into a routine application. With simplex only a fraction of the necessary experiments had to be carried out to reach the desired optimum.

10.7   DETERMINATION OF PENICILLINS

Penicillins and cephalosporins are hydrolyzed either by alkali treatment or by use of commercially available penicillinase. The hydrolysis product of penicillins is the corresponding penicilloic acid which may be measured directly or indirectly. In the presence of mercury(II) chloride, for example, penicilloic acid reduces molybdoarsenic acid to form a blue complex which is measured spectrophotometrically (9,10). The pH change caused by the formation of penicilloic acid can also be measured. The procedures will be outlined here based on either of these two detection principles.

Method 1: Determination of penicillin V in fermentation broths

This method is based on the work of Schneider (9).

Reagents

C   Penicillinase solution, 500,000 U/100 ml. Prepare the enzyme in a 100 ml buffer pH 6.2. The buffer is prepared as follows: dissolve 7.02 g citric acid and 23.55 g disodium hydrogen phosphate in 1 liter of distilled water, check the pH and sterilize the solution.

R1   Molybdoarsenic acid solution. For preparation of 1 liter: dissolve 30 g of ammonium heptamolybdate tetrahydrate in 400 ml distilled water, add 25.5 ml concentrated sulfuric acid and cool. Dissolve 3.6 g of disodium hydrogenarsenate

heptahydrate in a distilled water portion of about 25 ml and add this solution. Dilute with distilled water to a final volume.

R2   Dissolve 0.175 g mercury(II) chloride in 1 liter of water.

Manifold

Sample, S, is injected into a carrier stream, C, containing penicillinase, see Fig. 10.7. The two reagents, R1 and R2, are then added. R1 is a molybdoarsenic acid solution and R2 a mercury(II) chloride solution. The detection is made at 670 nm.
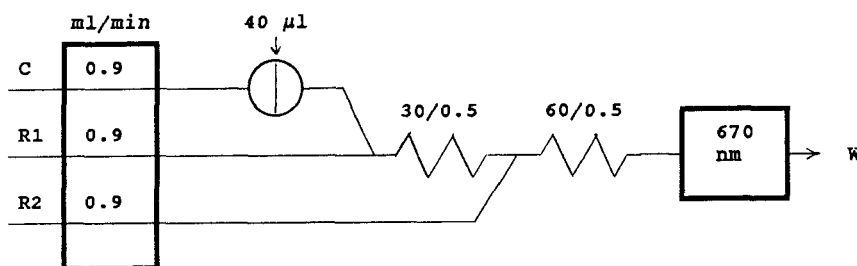

Fig. 10.7. Manifold for determination of penicillin V in fermentation broths. Numbers above coils denote coil length/i.d. (cm/mm).

Working range

Linear response is expected over the range 100 - 1000 units of penicillin V per ml.

Standard preparation

Dissolve a standard penicillin V salt in 25 ml citrate-phosphate buffer so that a 10,000 U/ml solution results. Dilute this stock solution with citrate-phosphate buffer to make a standard solution in the range 100 - 1000 U/ml.

Method 2: Determination of penicillins using immobilized penicillinase

This method was originally described in detail by Gnanasekaran and Mottola (11) and will only be outlined briefly here.

Penicillins G and V were determined in the range 0.05 - 0.50 mM using a simple FIA system comprised of a liquid delivery unit, an injection valve, an enzyme reactor and a glass electrode used as a detector. The carrier stream was a 1 mM phosphate buffer of pH 6.40. The carrier flow rate was 4 ml/min and the injection volume was 160 μl.

The enzyme reactors were of two types, namely glass capillary coils (1 m, 1.4 mm i.d.) and PTFE tubes (1.3 mm i.d.). Both were filled with etched and silylated glass beads. Leaching of the glass beads was performed with concentrated hydrochloric acid overnight. The beads were then washed with water, methanol and acetone and dried at 140 °C for 1 hour. Etching was performed with a saturated solution of ammonium fluoride/hydrofluoric acid in methanol. The methanol was evaporated in vacuum. Further treatment included heating to 450 °C for 3 hours, washing with methanol and leaching in hydrochloric acid as before. The silylation was performed with (p-aminophenyl)-trimethoxysilane. The glass capillary coil was treated in the same way as the glass beads before packing took place. The procedure for glutaraldehyde coupling entailed shaking of the silylated glass with a 2.5% solution of glutaraldehyde (20 ml) in 0.05 M phosphate buffer, pH 7, for 1 hour. Washing was made with distilled water. The immobilization procedure consisted of shaking 0.5 g of glass beads with 10 ml 0.05 M phosphate buffer and penicillinase (1700 U) at 4 °C for 4 hours. The glass was then washed successively with distilled water, 1.0 M potassium chloride, and 0.05 M phosphate buffer.

The detector was a combined glass electrode with a flat surface inserted vertically in a cell with a horizontal flow channel. The flow cell "volume" was about 50 μl.

10.8 DETERMINATION OF DRUGS WITH PHENOLIC STRUCTURE USING THE 4-AMINOANTIPYRINE METHOD

Phenol reacts with 4-aminoantipyrine (AAP) in an alkaline solution and in presence of potassium hexacyanoferrate(III) according to
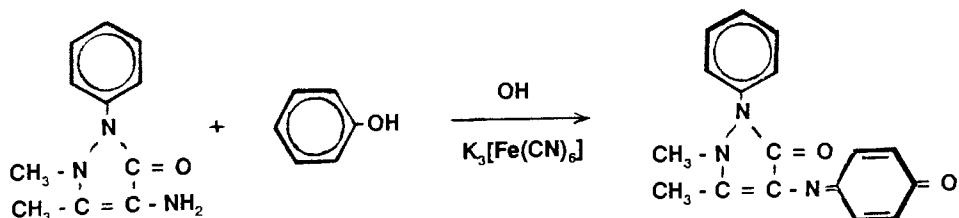
The reaction product absorbs strongly at 550 nm but it is unstable. Manual procedures have to take this fact into account. Consequently, the process for mixing sample and reagents must be carefully defined and several absorbance readings must be performed at preset times. Mechanization of the manual procedure using FIA would solve these classical mixing and timing problems associated with many batch methods. FIA offers, by its nature, a reproducible mixing and a reproducible reaction time for sample and reagents. The method described here is based on the work of Strandberg and Thelander (12).

<u>Sample preparation</u>

Make sample solutions with a final concentration of about 0.3 mM.

<u>FIA principle</u>

<u>Manifold</u>

The sample, S, is injected into a carrier stream, C, comprised of distilled water. The first reagent stream, R1, is a pH 9.5 buffer. The second reagent stream, R2, is a 4-aminoantipyrine solution and the third, R3, is a potassium hexacyanoferrate(III) solution. The detection is made at 550 nm. The FIA manifold is shown in Fig. 10.8.
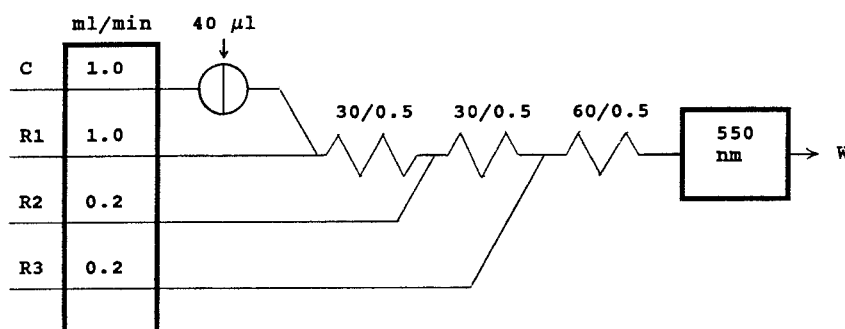
Fig. 10.8. Manifold for determination of drug compounds with phenolic structure. Numbers above coils denote coil length/i.d. (cm/mm).

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Reagents

C   Distilled water.

R1   0.3 M borate buffer, pH 9.5.

R2   0.25% 4-aminoantipyrine. Prepare freshly each day.

R3   1% potassium hexacyanoferrate(III). Prepare freshly each day.

Concluding remarks

As a general rule, 10-fold and 30-fold excesses of 4-aminoantipyrine and potassium hexacyanoferrate(III) respectively are recommended in comparison with the sample concentration. Large reagent concentrations might give rise to precipitation in the system. Potassium persulfate can, for certain applications, be considered as an alternative oxidant to potassium hexacyanoferrate(III). The persulfate reagent solution should then be alkaline with a pH value of about 1 liter. It is necessary to adapt both the manifold *design* and the composition of the reagent and carrier solutions to the desired application since derivative phenolic drug compounds may behave differently. The outlined procedure should, consequently, be regarded as a starting point in this development work.

REFERENCES

1.   B. Karlberg, P.A. Johansson and S. Thelander, Anal. Chim. Acta, 104 (1979) 21. [31]

2.   R.A. Salerno, C.O. Dell, N. Cyanovich, B.P. Bubnis, W. Morges and A. Gray, Anal. Biochem., 151 (1985) 309. [651]

3.   M. Jaffe, Z. Physiol. Chem., 10 (1886) 391. [-]

4.   D. Heinegard and G. Tiderstrom, Clin. Chim. Acta, 43 (1973) 305. [-]

5.   P.K. Jaynes, R.D. Feld and G.F. Johnson, Clin. Chem., 28 (1982) 114. [-]

6.   F. Winquist, I. Lundstrom and B. Danielsson, Anal. Chem., 58 (1986) 145. [676]

7.   Y. Bergquist, K. Hed and B. Karlberg, Int. J. Sports Med., 9 (1988) 73. [-]

8.   D. Betteridge, T.J. Sly, A.P. Wade and J.E.W. Tillman, Anal. Chem., 55 (1983) 1292. [310]

9.   K.A. Holm, Anal. Chem., 44 (1972) 795. [-]

10.   I. Schneider, Anal. Chim. Acta, 166 (1984) 293. [512]

11.   R. Gnanasekaran and H.A. Mottola, Anal. Chem., 57 (1985) 1005. [554]

12.   M. Strandberg and S. Thelander, Anal.Chim. Acta, 145 (1983) 219. [268]

CHAPTER 11

FOOD AND FEED

11.1   INTRODUCTION

The two most difficult aspects of food and feed analytical chemistry are the complex sample matrix and the heterogeneous nature of the sample. The majority of the samples are solids, like grains, fruits, vegetables and meats. Because of the complex nature and heterogeneous nature, a comprehensive sample pretreatment is critical to the analytical procedure. Grinding, mixing, and dissolution are the usual steps of the sample preparation. As of now these steps are not easily handled by FIA. However, after dissolution the sample still requires additional sample preparation. Usually it is necessary to attempt to remove the analyte of interest from the dissolution solution, to chemically mask the potential interferents, or to dilute the sample. All three of these operations can be routinely performed using FIA.

For many analyte/samples there are standard methods. In many cases, the standard water methods are applicable for food and feed analysis. Unfortunately, the food and feed sample matrix many times limits the reaction rates. The combination of sample pretreatment and analytical detection may also slow the apparent reaction rates. More than any other application area, food and feed methods require heating and long reaction times, 15 minutes or more. For such methods FIA is not the best continuous flow system to use.

The following sections are selected examples which will serve to elucidate the different possibilities which FIA offers. Knowledge of the chemistry and the amount of imagination are the only limiting factors for the development of other FIA based methods.

11.2   DETERMINATION OF STARCH IN FOOD AND FEED PRODUCTS

Quantitative determination of starch is important and often requested for food and feed products, in particular for grains. Starch, which is a mixture of linear and branched glucose polymers, is first hydrolyzed to oligosaccharides by incubation in the presence of a thermostable enzyme. By further treatment with different enzymes, hydrogen peroxide is finally obtained and is detected spectrophotometrically after enzymatic reduction in the presence of a chromogenic reagent. Several of the enzymatic reactions

which are required to accomplish the entire determination can be performed in an FIA system provided with reactors containing immobilized enzymes (1).

Sample pretreatment procedure

Grind the sample to a particle size of less than 0.5 mm. Weigh accurately 40 - 50 mg of the sample into a 30 - 50 ml glass vessel provided with a stopper. Add 25 ml 0.05 M acetate buffer, pH 5.0, also containing 280 mg calcium chloride per liter. Add 0.1 ml thermostable α-amylase (e.g. Termamyl 240 Knu/g, Novo, Denmark) and heat to 95 °C. Shake 2 - 3 times during 30 minutes. Cool and centrifuge. The sample solution extract should contain 0.01 - 0.3 mg starch/ml.

FIA priciple for starch determination

The liquid sample extract, S, obtained according to the procedure described above, is injected into a buffer stream, C, and passes through a series of reactors containing immobilized enzymes, see Fig. 11.1.
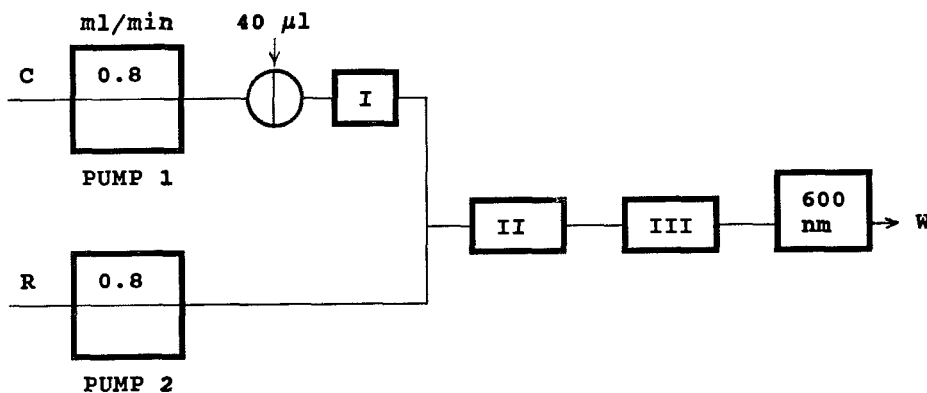
Fig. 11.1. Manifold for determination of starch in food and feed products.
I = amyloglucosidase, II = glucose oxidase/mutarotase, and III = peroxidase reactors.

The first reactor contains amyloglucosidase which catalyzes the hydrolysis of the starch to free glucose. Reactor II contains two immobilized enzymes, namely glucose

oxidase and mutarotase. Here, the glucose is oxidized to gluconic acid and hydrogen peroxide. The added reagent, R, is a mixture of chromotropic acid and 4-aminophenazone. Reactor III contains immobilized peroxidase. The colored reaction product is measured at 600 nm.

Reagents

All reagents must be degassed before use.

C   Citrate-phosphate buffer, pH 4.5. Prepare by addition of 0.2 M citric acid to a 0.2 M disodium hydrogen phosphate solution until a pH value of 4.5 is obtained. Dilute 125 ml of this solution to 1000 ml with distilled water.

R   2 mM chromotropic acid + 1 mM 4-aminophenazone in citrate-phosphate buffer. The buffer is prepared as above but is adjusted to pH 7.0. This reagent is should be prepared daily and kept in a dark bottle.

Enzyme preparation

Use enzymes with average or high activity. Dissolve the enzymes in cold 0.1 M pH 7.0 - 8.5 phosphate buffer (30 mg/ml) and dialyze against 1 liter of the same buffer at 4 °C during a couple of hours.

Preparation of CPG (glutaraldehyde) for reactors I and II

Use controlled pore glass (CPG) with a hydrophobic surface, CPG-10, 0.075 - 0.125 mm (Serva).

1. Boil 10 ml CPG in 150 ml 5% nitric acid for one hour.
2. Wash the CPG on a G3 filter with 1 1 of water. Let the CPG dry at 95 °C.
3. Prepare a 10% aminopropyltrietoxysilane solution (5 g + 45 ml of water) and adjust the pH to 3.45 by adding 5 M hydrochloric acid.
4. Add the dry CPG to this solution and let stand at 75 °C in a water bath for 3 hours. Swirl the storage vessel each 15 minutes.
5. Wash the CPG on a G3 filter with 1 liter of water and dry at 95 °C overnight. CPG prepared in this way can be stored for later use.
6. Prepare a 2.5% glutaraldehyde solution in 0.1 M phosphate buffer, pH 7.0 - 8.5. Add 1.0 ml of this solution to each 100 mg of CPG.

7.   Let the mixture stand for one hour at room temperature and under reduced pressure during the first 30 minutes. Wash on a G3 filter using cold water.

Column dimensions

The columns should be made of 2.2 mm i.d. PTFE tubing with screw fittings. Plugs of glass wool should be used to prevent gel leakage. Column dimensions: reactor I, 100 mm long and 360 μl volume; reactor II, 75 mm long and 270 μl volume; and reactor III, 50 mm long and 180 μl volume.

Column preparation, reactor I and II

Transfer the cold enzyme solutions to the washed and wet glutaraldehyde-activated CPG. Store the suspensions overnight at 4 °C. Wash first with 250 ml cold 0.1 M phosphate buffer, pH 7.0 - 8.5, and then with 500 ml cold distilled water on a G3 filter.

Reactor I - 60 mg amyloglucosidase/g CPG.
Reactor II - 2.5 mg mutarotase and 25 mg glucosoxidase/g CPG.

Column preparation, column III

Use diazo activated CPG (SP 500 diazoniumphenyl 0.04 - 0.1 mm, Serva). Mix 100 mg CPG with 0.5 ml cold phosphate buffer (0.1 M, pH 8.9) and add the cold enzyme solution (60 mg/g CPG). Reduce the pressure for 5 minutes and let the solution stand at 4 °C overnight. Wash on a G3 filter with cold phosphate buffer, pH 8.9. Pack the column.

Preparation of standard solutions

Weigh about 0.05 g starch and correct for the amount of dry matter. Add 25 ml 0.05 M acetate buffer, pH 5.0, containing 280 mg calcium chloride per liter. Add 0.1 ml thermostable α-amylase. Heat to 95 °C and keep the mixture at the same temperature for about 30 min. Shake the mixture intermittently. Cool and centrifuge. Make a set of calibration solutions by taking aliquots of the stock solution. Dilute each aliquot to 100 ml with distilled water. The concentration range should be 0.01 - 0.3 mg starch/ml in the final standard solutions. The calibration graph should be linear.

11.3 DETERMINATION OF CAFFEINE IN COFFEE AND TEA

Caffeine in coffee and tea samples is readily determined after a liquid-liquid extraction procedure. The caffeine itself absorbs light in the UV range; a wavelength of 276 nm is recommended. The extraction is performed at a relatively high pH value to prevent co-extraction of undissociated acid compounds in the sample. Chloroform has proven to be a useful organic solvent for the extraction.

<u>Sample preparation</u>

Roasted coffee: 1 g dissolved in 500 ml hot water
Soluble coffee: 1 g dissolved in 1000 ml water
Decaffeinated coffee: 1 g is dissolved in 100 ml water
Tea: Treat the sample with hot water. Amounts and volumes have to be adapted to the brand since both the water content and the caffeine concentration in the leaves might vary considerably. Filtration of the samples might become necessary.

<u>FIA priciple for determination of caffeine</u>

<u>Manifold</u>

The manifold is shown in Fig. 11.2.
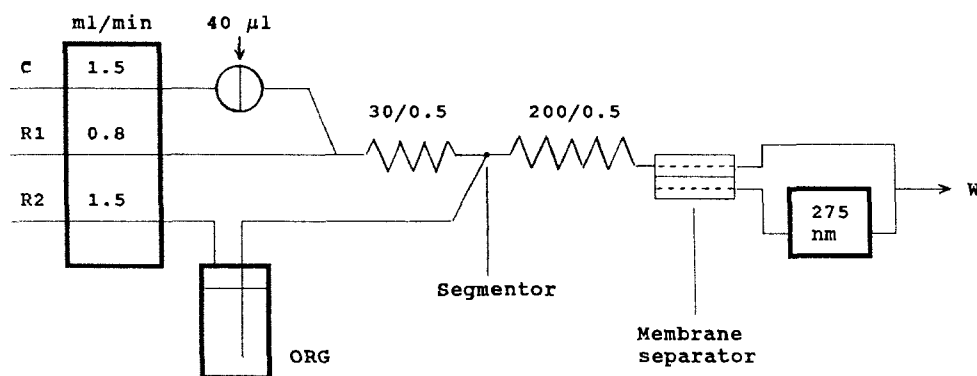
Fig. 11.2. Manifold for the determination of caffeine in coffee and tea.
ORG = chloroform. Numbers above coils denote coil length/i.d. (cm/mm).

The sample, S, is injected into a carrier, C, comprised of distilled water and merged with an alkaline buffer, R1. Water, R2, is pumped into the displacement bottle containing chloroform, Org. The aqueous and the organic streams merge at the segmentation point denoted by "segmentor" in Fig. 11.2. The extraction takes place in a PTFE coil, length 200 cm, i.d. 0.5 mm. The two phases are separated using a membrane separator and the organic phase is led to the flow cell of the UV detector. The absorbance is measured at 270 nm.

Carrier and reagents

C   Distilled water.

R1   Ammonia diluted 1:10 with water.

R2   Distilled water.

Preparation of standard solutions

Prepare aqueous caffeine standards in the range 1 - 7 mg/l.

Concluding remarks

The repeatability should be better than 1% r.s.d. and the sample throughput is typically 60/h.

11.4   OXIDIZED KETONE BODIES IN MILK

Dairy cattle in their peak lactation period have very sensitive energy balances. Undernutrition often leads to increased levels of ketone bodies in the blood and, at the same time, increased excretion of ketone bodies. Very high levels of ketone bodies indicate the clinical disease ketosis, while lower but higher than normal levels may be regarded as indicators of an energy imbalance in the diet. It is, therefore, important to detect ketone bodies as a basis for correction of diet even in apparently healthy cows.

The term ketone bodies normally includes 3-hydroxybutyric acid, acetoacetic acid and acetone. The sum of the acetoacetic acid and acetone is referred to as oxidized ketone bodies. These concentrations of oxidized ketone bodies in the blood stream are considered as accurate indicators of energy imbalance (2).

Acetoacetate in blood can be determined enzymatically using 3-hydroxybutyrate dehydrogenase with the nicotinamide adenine dinucleotide (NADH) as a coenzyme. Either ultraviolet absorption (3,4) or fluorescence (5,6) can be monitored. Unfortunately, these methods are not directly applicable to a sample matrix like milk. The low opacity of milk makes spectrophotometric methods useless. A rapid semiquantitative test strip method based on disodium pentacyanonitrosoylferrate(II) is available, but it is not selective or sensitive enough to be used except as a screening test. Acetoacetic acid is easily decarboxylated to acetone which can be determined with many colorimetric reagents (2-13). Once again the milk matrix precludes the utilization of this procedure without some type of separation step. Distillation, extraction, diffusion and chromatography have all been used. These procedures are extremely time consuming and tedious.

FIA principle for determination of ketone bodies

The conversion of acetoacetic acid to acetone by thermal decarboxylation is a good method to estimate the oxidized ketone bodies. The decarboxylation is carried out at low pH and 100 °C for 35 min. The problem is the milk matrix. Since acetone is volatile and gas diffusion is a viable technique in FIA, it was possible to develop a FIA method for oxidized ketone bodies (14).

The sample is pretreated for the decarboxylation step external to the FIA system. The treated sample is then injected into the donor stream, pH 6.9 - 7.0 phosphate buffer (0.1 M), of a gas diffusion manifold. The gas diffusion membrane is a 0.07 mm thick PTFE teflon membrane. A portion of the acetone in the milk transports across the membrane into an acceptor stream which contains hydroxylamine/methyl orange reagents, 0.0435 M/7.6×10^{-5} M with 2×10^{-4} M NaOH. The pH of the acceptor stream should be about 3.5 - 4.0. The acceptor stream reagent is useful for no longer than one week due to the decomposition of the hydroxylamine.

The diffused acetone reacts with the hydroxylamine to form acetoxime. This reaction creates a shift in pH which is monitored by the methyl orange indicator. The wavelength of observation is 520 nm. The manifold used for this determination is shown in Fig. 11.3.
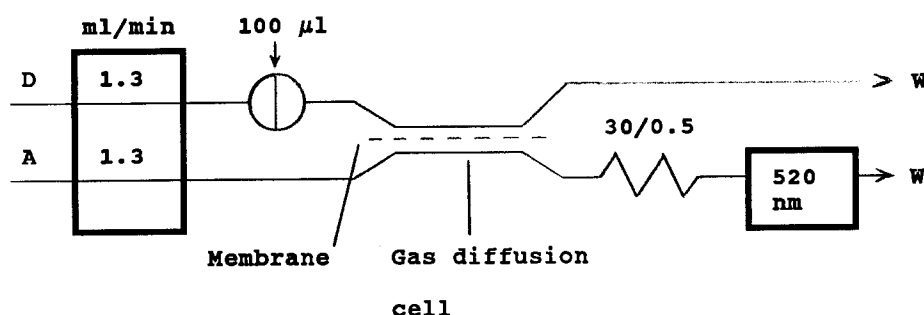
Fig. 11.3. Manifold for the determination of oxidized ketone bodies. Numbers above coil denote coil length/i.d. (cm/mm). D = donor stream, A = acceptor stream.

The sample pretreatment deserves some attention. Thermal decomposition using perchloric acid requires 15 minutes, but also denatures the proteins causing blocking of the tubing. Simple heating takes 35 minutes but changes the fat structure. The fat particles accumulate on the diffusion membrane thereby changing the membrane properties. Mechanical homogenation of the sample after heating eliminates this problem.

Interferences

Ammonia, formaldehyde and methyl ethyl ketone can also diffuse through the membrane and will, therefore, affect the reagent stream pH. A 10 mM formaldehyde solution gives a peak equal to 6% of a peak produced by a 1 mM acetone solution. A solution of 1 mM methyl ethyl ketone gives about 77% of a peak produced by 1 mM acetone.

Detection limit, accuracy and precision

The detection limit is 0.1 mM acetone at 4 times signal to noise ratio. The accuracy is between 2 - 5% relative. The precision is between 0.5 - 2% r.s.d.

11.5   SULFITE AND SULFUR DIOXIDE IN BEVERAGES

The compounds sulfur dioxide and sulfite ion are used in the food industry as preservatives. The acid base equilibrium between sulfur dioxide and sulfite ion is:

$$H_2O + SO_2 \rightleftharpoons H_2SO_3 \rightleftharpoons H^+ + HSO_3^- \rightleftharpoons 2H^+ + SO_3^{2-}$$
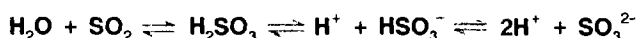
It is essential that either free sulfite or total sulfite be determined. The chemistry used in the determination is simple, but the sample matrix can introduce additional complications. The actual species determined in both cases is sulfur dioxide. Reaction chemistries which are relatively selective for sulfur dioxide exist, whereas reagents for sulfite ion are not considered selective.

The determination of sulfite ion in beverages is based on analyte conversion to sulfur dioxide accomplished by shifting the acid-base equilibrium towards the acidic side using a mineral acid. The sulfur dioxide is usually determined using the reaction product of sulfur dioxide, formaldehyde and pararosaniline. In the batch mode this method can be used to determine sulfur dioxide and converted sulfite ion with the existing sulfur dioxide, the difference between the values obtained in these two determinations being the original sulfite ion concentrations. Determination by difference might adversely affect the accuracy. The difference value is usually a small number while the two values used to obtain this difference can be large. Propagation of error will sometimes cast doubts about the validity of the value determined by difference.

Upon initial inspection it would appear that this method would be easily adapted to FIA. Other than throughput and precision, would there be any other potential advantages? The reaction between pararosaniline and sulfur dioxide is slow. When this chemistry is run in an FIA system the reaction is not complete by the time the sample zone passes through the detector. However, since the residence time of the FIA system can be kept perfectly constant this is not a stumbling block.

If increased detectability is a goal stopped flow can be utilized to increase residence time and, therefore, allow the reaction to proceed to completion. The overall lowering of the detection limit may not be worth the time. But what if the sample matrix is a problem for the method? Stopped flow can still be utilized. In one reported example, the use of stopped flow circumvented the problem of high background absorbance of the sample (15). If the beverage is red wine, the red color produced by the reaction between pararosaniline and sulfur dioxide is obscured by the wine color. Different red wines contribute differently. By utilizing stopped flow the pararosaniline sulfur dioxide reaction continues to produce red color and this contribution is observed as an increase in the absorbance, see Fig. 11.4.
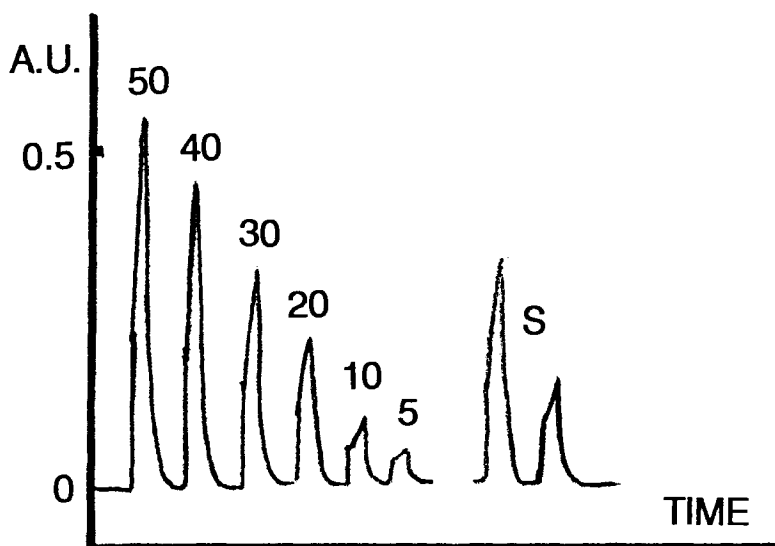
Fig. 11.4. Typical signals obtained for the determination of sulfur dioxide in red wine using a stopped flow FIA technique (15). The stopped time is 15 seconds. Numbers at peaks denote concentrations in ppm. S = samples.

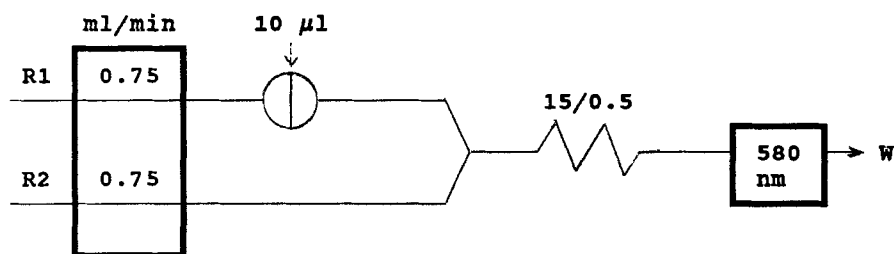The manifold used to produce the results in Fig. 11.4 is shown in Fig. 11.5.

Fig. 11.5. Manifold for the determination of sulfur dioxide in wine based on a stopped flow FIA technique (15). Numbers above coil denote coil length/i.d. (cm/mm).
R1 : 0.08% pararosaniline in 0.3 M sulfuric acid. R2 : 0.5% formaldehyde in 0.3 M sulfuric acid. The two streams are stopped shortly after the peak maximum is reached. The stopped time is 15 seconds.

The calibration of the system is based on the amount of signal produced during the stopped flow period. A variety of calibration curves with varying sensitivities can be constructed since any point along the descending portion of peak can be used. There are only two basic constraints on the system and they are both based on the pump system. The timing of the stop and start period must be absolutely reproducible and when the pump stops it must stop immediately with no momentum.

There is one more approach which is used primarily to enhance selectivity and that is gas diffusion. In this case, "free" sulfite is converted to sulfur dioxide in the FIA system. The formed sulfur dioxide diffuses across a gas permeable membrane into an acceptor stream of formaldehyde and pararosaniline. The "total" sulfite content in the sample can then be measured using the same manifold if the sample is pretreated according to the batch method procedure. Usually the sample is pretreated with sodium hydroxide. The sample is then injected in a carrier stream which is merged with a mineral acid stream prior to the gas diffusion step in order to convert all sulfite to sulfur dioxide. The gas diffusion manifold creates selectivity by only allowing volatile species to transport to the acceptor stream. Matrix interference such as the high absorbance background of red wine is completely avoided. A typical manifold is presented in Fig. 11.6.
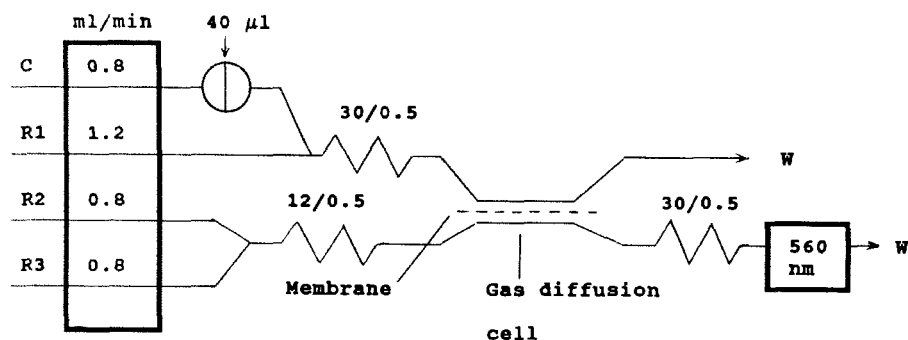
Fig. 11.6. Manifold for the determination of "free" sulfur dioxide using gas diffusion. Numbers above coils denote coil length/i.d. (cm/mm). C : distilled water, R1 : 0.3 M sulfuric acid, R2 : 0.74% formaldehyde solution (aqueous), R3 : 0.08% pararosaniline acetate solution (aqueous).

The two described procedures, stopped flow and gas diffusion, can be combined to produce a selective and sensitive method. In this design, a large volume of the sample, 350 μl or more, is injected into a continuously pumped carrier (donor) stream while the acceptor stream containing pararosaniline is under stopped flow conditions. Sulfur dioxide will continuously be transported across the membrane as long as sample is present in the gas diffusion manifold and as long as the reagent capacity is sufficient to allow a complete consumption of the diffused gas. The peaks obtained are more symmetric in shape than the usual FIA peaks. Typical calibration ranges for these reactions are 2 - 300 mg/l calculated as sulfur dioxide. Reproducibility is usually less than 1% r.s.d.

The most appropriate method for the determination of free sulfite in wines seems to be the malachite green method (16). As stated earlier, improvements in accuracy can be realized by minimizing the amount of sample pretreatment. The use of FIA certainly achieves this goal. The injected sample is exposed to strong citric acid at pH 2 which in turn liberates SO_2 gas from free sulfite and the labile portion of bound sulfite. Once again, the gas diffusion technique using a teflon membrane is used to separate the SO_2 from the sample matrix. The SO_2 is transported onto an acceptor stream of malachite green dye buffered to pH 8. The SO_2 discolors the dye. This discoloration is proportional to the SO_2 transported accross the membrane (16). The manifold is shown in Fig. 11.7.
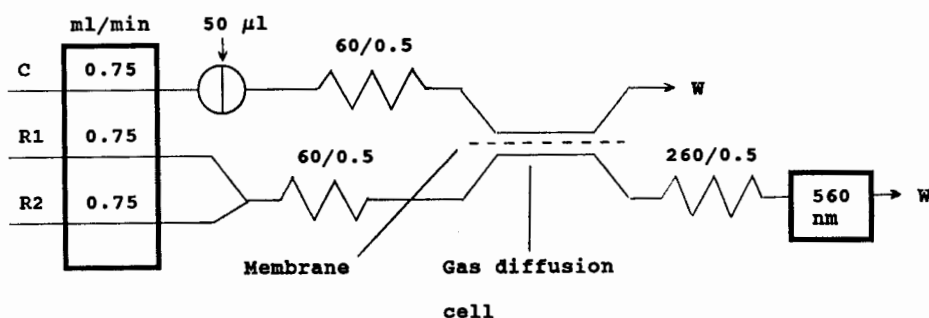
Fig. 11.7. Manifold for the determination of SO_2 in wines using malachite green. Numbers above coils denote coil length/i.d. (cm/mm).

The procedure varies in terms of sample preparation. In the case of wines, citric acid solution of pH 2 is used. For foods like pineapple or shrimp extraction or digestion procedures must be used (16).

Reagents

The stock phosphate-buffered malachite green solution is prepared as follows: Dissolve 200 mg malachite green (99%) and 8.5 g of potassium dihydrogen phosphate in about 900 ml of water and dilute to 1 liter. This solution is filtered through a 0.45 μm cellulose acetate membrane filter and stored at 4 °C. All dilutions of this solution are made with distilled water.

C   0.15 M sulfuric acid. Add 8.3 concentrated acid to about 900 ml distilled water, mix and dilute to 1 liter.

R1   Malachite green reagent: Dilute the stock phosphate-buffered malachite green solution 1 + 9 with water. Prepare fresh daily.

R2   0.094 M phosphate buffer. Dissolve 16.36 g of potassium monohydrogen phosphate in about 900 ml of water and dilute to 1 liter.

Preparation of standard solutions

The sulfite standard solutions can be preserved either by tetrachloromercurate (2.35 g NaCl + 5.45 g $HgCl_2$ per liter) or acetaldehyde (1%). Standard solutions are made in the range 0 - 20 ppm using Na_2SO_3.

Rinsing procedure

Wash out the FIA system by pumping ca 20 ml 0.02 M NaOH solution through all lines. Keep this solution in the pump tubes and in the manifold overnight but release, as usual, the compression band or cam of the pump so that the pump tubes are note flattened.

Results

The results of this method are a linear range from 1 - 20 ppm as SO_2 with a detection limit of 0.1 ppm. The analysis time is 1 minute. The observed r.s.d. is 1%. When this method is compared against the pararosaniline, colorimetric extraction, and enzymatic methods the results are in good agreement (16).

11.6 DETERMINATION OF POTASSIUM BROMATE IN FLOUR

Potassium bromate is added to flour to improve the breadmaking qualities. The maximum allowed level in bread may vary but is typically 50 mg/kg of flour mass (17). The official method is based on iodometry. Potassium iodide is added to an acidic sample solution and iodine is formed. Sodium thiosulfate is added in excess and the excess is titrated with potassium iodate. The FIA method described here is based on a colorimetric principle of determining the iodine formed after the addition of potassium iodide to the sample (17). For each bromate ion three iodine molecules are formed provided that iodide is added in large excess and that the solution is acidic.

Ammonium molybdate is used to increase the iodine formation rate. Iodine and starch form the colored complex. However, the color intensity varies with time. Corresponding batch methods may produce inconsistent results due to individual working modes among different analysts. These differences do not exist with the FIA methodology.

Sample preparation

Weigh 5 g of flour into a 100 ml screw-capped bottle and add 20 ml 2% zinc sulfate solution. Shake the bottle to disperse the flour. Add 4 ml sodium hydroxide solution, concentration 21 g/l. Add 1 ml distilled water. Shake the bottles for 5 min on a wrist-action shaker. Centrifuge for 5 min at 1150 x g. Filter the supernatant through a Whatman no. 4 paper and use the filtrate to fill the injector of the FIA system.

FIA principle

The sample, S, is injected into a zinc sulfate/starch solution carrier, C, and merged with a reagent solution, R1, comprised of ammonium molybdate. After mixing in a 30 cm coil, i.d. 0.7 mm, a potassium iodide reagent solution, R2, is added. The detection wavelength is 570 nm. The manifold for determination of potassium bromate is shown in Fig. 11.8.

Reagents

C   The carrier solution is prepared from 0.45 g zinc sulfate, 0.59 g of anhydrous sodium sulfate and 25 ml of starch solution. The latter solution is prepared by mixing 1.0 g of soluble starch to a creamy paste with a little water and pouring the paste in 100 ml of boiling water. The starch solution mush be prepared fresh every few days.

R1   Ammonium molybdate in sulfuric acid. The ammonium molybdate concentration should be 0.3 g/l in sulfuric acid diluted 1 to 9 with distilled water.

R2   Potassium iodide solution, 10 g/l. This solution is prepared in distilled water with a few drops of sodium hydroxide added to prolong the shelf life.
The closing down procedure of the FIA system should include a flush cycle for all lines using 2% sodium hydroxide first and then distilled water. Pump each solution 2 - 3 min.
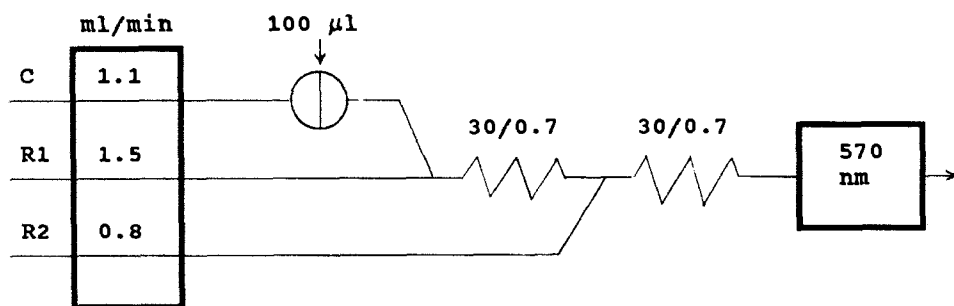
Fig. 11.8. FIA manifold for determination of potassium bromate in flour. Numbers above coils denote coil length/i.d. (cm/mm).

Preparation of standards

Make a stock solution of 0.2500 g potassium bromate per liter. Prepare working standards in the range 25 - 125 mg/l. Weigh 5 g untreated and unbleached flour into a 100 ml screw-capped bottle and add 20 ml 2% zinc sulfate solution. Put on the cap and shake to disperse the flour. Open and add 4 ml sodium hydroxide, concentration 21 g/l, and 1 ml of the working standard solution. Shake, centrifuge and filter as described previously for the sample. Use distilled water instead of working standard to prepare a blank.

Interferences

Oxidizing or reducing agents present in the flour sample may interfere.

11.7   SELENIUM IN BIOLOGICAL MATERIAL

The determination of selenium in biological material requires a method which can work in the presence of other metal ions and some interfering anions. The conventional way to determine selenium is to use atomic absorption, AA, or inductively coupled plasma optical emission spectroscopy, ICP. Direct aspiration of samples containing selenium into the flame and plasma has not been successful most often because the concentration levels are low. Furthermore, the excitation and emission lines are in the vacuum and near vacuum ultraviolet region which means that many sample constituents may interfere.

As a rule, hydride generation is the analyte conversion step that is performed to address the abovementioned problems. In this technique the hydride forming species are converted to volatile hydride compounds. Elements like arsenic, antimony, tin and selenium are easily determined using this procedure. The sample containing the hydride forming species is usually mixed with hydrochloric acid and sodium borohydride, although some researchers have used different acids and hydrogen producing compounds. The hydrogen produced reacts with the metal ion to form the metal hydride. For example, in the case of arsenic, the hydride is arsine, AsH_3. The volatile metal hydride is purged from the solution and collected, a type of preconcentration step, before the hydride is allowed to enter the flame or plasma. Clearly, the reproducibility of this procedure depends on timing.

However, the accuracy of this method is also dependent on whether the potential interferents are eliminated from the sample. The hydride generation methods have two types of interferences, atomizer and chemical. The atomizer interferences include the problems generated by the atomization process and the thermal processes in the flame or plasma. As a rule, the FIA system will not affect the atomizer interferences. If the Dedina cell is employed many of the atomizer interferences are minimized (18).

The chemical interferences can be categorized into two groups, transition metals and other hydride forming ions. The transition metal interferences appear to be due to the fact that the transition metal ions are in higher concentration than the hydride forming elements and that the transition metal ions form their own hydrides. The transition metal hydrides exhibit low solubility and will precipitate. These particles adsorb the hydrides formed by the selenium, arsenic, etc. In this case the obvious problem is that not all the selenium hydride formed reaches the detector. In addition, the selenium hydrides will decompose to metallic selenium in some systems.

The other chemical interferences are even more puzzling. Arsenic will affect the amount of selenium hydride observed and selenium will affect the amount of arsine observed. All the volatile hydride forming elements will interfere with the determination of other volatile hydride forming elements. The exact mechanism is not known. Even speculation is at a minimum. It should be clear that the determination of any of the

volatile hydrides is difficult because of the numerous interferences and the irreproducible manner in which the generation and trapping of the hydrides is handled. Continuous flow analysis would most certainly improve the precision of these methods.

There are three approaches to automating the generation of hydrides:
1) mechanization of the manual mixing of the sample and the reagents and placing the mixture in a gas/liquid separator, 2) use of FIA or segmented flow to accomplish the mixing of the sample and the reagents and contain the gases in the stream, and 3) use of FIA and dual phase gas diffusion to separate the volatile hydride from the sample matrix before some of the interfering reactions can occur.

For the first two options some type of gas/liquid separator must be used. Such separators are commercially available from most manufacturers of AAS equipment.

Fig. 11.9 shows a manifold based on the principle of dual phase gas diffusion.
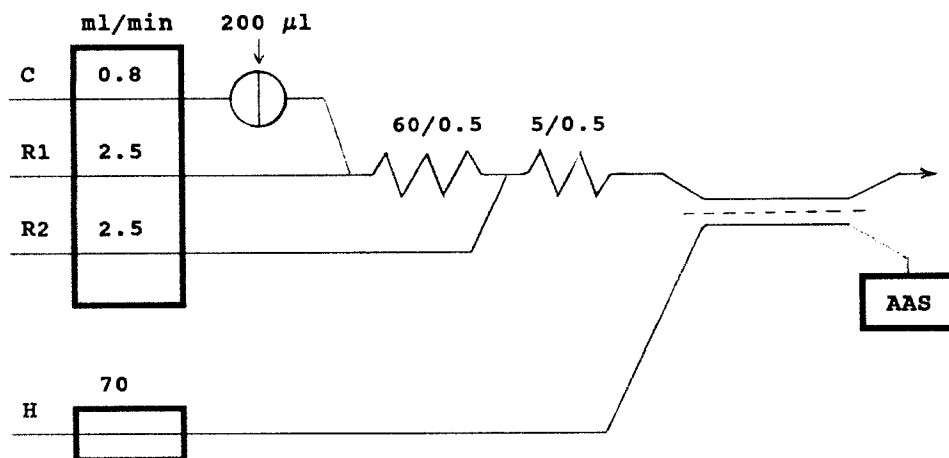
Fig. 11.9. Manifold for dual phase gas diffusion. Numbers above coils denote coil length/i.d. (cm/mm).

Reagents and standard solutions

There are several alternatives to the reagent selection, but the reagents given here are the most popular. Sodium tetrahydroborate(III) solution is 2% (w/v) in $NaBH_4$ and 1% (w/v) in potassium hydroxide and must be prepared fresh daily. Selenium standards of 1000 mg/l are prepared from sodium selenite.

Performance

In addition to interferences, the biggest problem with automated hydride systems has been the noise generated by the separator. This type of separator produces a series of spikes in the baseline every time a bubble is released from the system.

Interferences are usually dealt with by using two chemical tricks. With potassium iodide added to the reaction, the lowest valence state of the volatile hydride forming elements will be presented to the sodium borohydride. This is important since there are differences in the rates of reaction for the different valence state. The second trick is to use hydroxylamine hydrochloride to reduce the transition metal ion interference. The addition of these reagents has been given as a reason to incorporate segmented flow, reduced sample dispersion, instead of FIA (19). Although the hydroxylamine hydrochloride step does significantly reduce the transition metal ion interference, it makes the procedure more complicated and becomes more dependent on the sample matrix remaining constant. By taking advantage of the kinetic discrimination potential of FIA the hydroxylamine step is unnecessary since the transition metal ions cannot form their hydrides as fast as the volatile hydride elements. This is accomplished by immediate removal of the volatile hydride from the system. The adsorption process does not have time to occur. For example, copper(II) interferes at 500 weight percentage in a conventional FIA system while the dual phase gas diffusion system does not exhibit interferences even at a 10,000 weight percent interferent to analyte ratio.

None of the systems completely eliminates the interferences created by the other volatile hydride forming elements. Surprisingly, the different systems for selenium give different degrees of interference for the same species, e.g., Te(IV) 100, 10, and 25 weight percents for the normal FIA, segmented flow and dual phase gas diffusion, respectively.

Improved selectivity for the individual volatile hydride forming elements can be achieved by varying the concentrations of the reagents and some of the reagents themselves. It is important to check the actual selectivity of the system being used before the determinations are evaluated. For most samples there is little concern over the other volatile hydrides.

REFERENCES

1.   S. Bengtsson, K. Larsson and I.L. Orde, personal communication, 1988 (National Laboratory for Agricultural Chemistry, Uppsala, Sweden). [-]

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2.   H. Steger, H. Girschewski, B. Piatkowski and J. Voigt, Arch. Tierernahr., 22 (1972) 157. [-]

3.   D.H. Williamson, J. Mellanby and H.A. Krebs, Biochem. J., 82 (1962) 90. [-]

4.   K.G.M.M. Alberti and T.D.R. Hockaday, Br. Med. J., 2 (1972) 565. [-]

5.   C. Olsen, Clin. Chim. Acta, 33 (1971) 293. [-]

6.   P.T. Ozand, R.L. Hawkins, R.M. Collins Jr., J.T. Tildon and M. Cornblath, Biochem. Med., 14 (1975) 170. [-]

7.   H. Goshke, Clin. Chim. Acta, 28 (1970) 359. [-]

8.   H.G. Schuster and G. Baasch, Z. Med. Labortech., 12 (1971) 312. [-]

9.   C. Thin and A. Robertson, Biochem. J., 51 (1952) 218. [-]

10.   E. Mehnert, Arch. Exp. Vetrinarmed., 24 (1970) 1269. [-]

11.   A.D. Woolhouse, N. Z. J. Med. Lab. Technol., 25 (1971) 82. [-]

12.   V.H. Peden, J. Lab. Clin. Med., 63 (1964) 332. [-]

13.   H. Steger and J. Voigt, Arch. Tierernahr., 20 (1970) 631. [-]

14.   P. Marstorp, T. Anfalt and L. Anderson, Anal. Chim. Acta, 149 (1983) 281. [412]

15.   J. Ruzicka and E.H. Hansen, Anal. Chim. Acta, 114 (1980) 19. [71]

16.   J.J. Sullivan, T.A. Hollingsworth, M.W. Wekell, R.T. Newton and J.E. Larose, J. Assoc. Off. Anal. Chem., 69 (1986) 542. [788]

17.   B.G. Osborne, Analyst, 112 (1987) 137. [1285]

18.   J. Dedina, Anal. Chem., 54 (1982) 2097. [-]

19.   H. Narasaki and M. Ikeda, Anal. Chem., 56 (1984) 2059. [444]

CHAPTER 12

PROCESS AND QUALITY CONTROL

12.1   INTRODUCTION

Most of the time process chemistry falls under the general term of process control. However, process chemistry has three different functions: process analysis, process monitoring, and process control.

Process analysis is the technique of analyzing what actually happened in the process. Process monitoring is the determination of the analyte(s) of interest in order to evaluate what is happening.   While process control actually takes the information produced by the process monitoring and uses some type of artificial intelligence to decide on a course of action to correct the process conditions.

A second set of nomenclature consist of: on-line, at-line, off-line, in-line, and noninvasive. On-line refers to an analytical system which takes a sample directly from the process stream and immediately begins the determination of the analyte.   Off-line measurements are made on samples from the process stream which are taken to a laboratory. At-line measurement are made on samples which are removed from the process stream and the analytical method is performed at the location of the sampling. In-line analytical methods have the detector in the process system.   The noninvasive system directly measures the analyte of interest without physical sampling.

In the case of FIA, noninvasive type systems are, by definition, excluded. In-line systems are also unlikely but theoretically possible. An FIA system can be used in a laboratory, off-line, in the plant setting, at-line, and can be tied into the process stream, on-line.   The advantages of using FIA for process monitoring are: rapid change of methods, large throughput, real time data acquisition, and possibility to perform matrix modification. The latter two advantages are the most pertinent to process monitoring. Real or near real time data acquisition is critical to the efficiency of the process control system. The sooner a problem is identified, the sooner the control system can correct the problem. Matrix modification is important in the development of the analytical method. The usual process stream has a matrix which makes it difficult to selectively measure the analyte. An FIA system can incorporate matrix modification techniques which minimize or eliminate the matrix problems.

FIA is a tool to do chemistry. The expected FIA characteristics of high precision, high throughput, comparable detection limits and the potential for improved accuracy are

all met in the process system. The FIA system can be used to automate existing methods or to slightly modify and improve existing methods. More importantly, the FIA system can utilize selectivity enhancement, kinetic discrimination, and matrix optimization to create new methods which are not possible in the batch or segmented continuous flow systems.

Before discussing the hardware aspects of FIA process monitors, a short discussion on sampling is necessary. The majority of the time the process stream is not homogeneous. Therefore, sampling is a critical problem. FIA will not improve the sampling capabilities of an analytical system. It will, however, provide the means to acquire larger data sets which will permit greater statistical confidence in the results.

12.2   FIA COMPONENTS IN PROCESS CONTROL

The typical FIA system contains a pumping device, a sample introduction device, reaction manifold(s), and a detector. Connecting these components is some type of tubing. The tubing materials which has been used to date are teflon, stainless steel, and to a lesser extent other polymers. Stainless steel is used primarily because of its ruggedness. The negative point about stainless steel is that the analyst cannot see through the tubing as is in teflon. This makes trouble shooting more difficult to perform. A second problem for stainless steel is that some FIA techniques, such as extraction, will not work for aqueous samples since the mechanism requires that an organic film is formed on the walls, (i.e. the tubing must be wettable by organics). Other than those constraints, stainless steel is a good choice.

Teflon tubing is rigid and reasonably rugged. The chemistry is easily observed since the teflon is transparent enough to see through. The negative to teflon can be in situations where there is a large amount of protein in the stream. Teflon will adsorb protein to its surface. If this surface coat is undesirable, then teflon is clearly a bad choice for tubing material.

Another tubing parameter to be considered is the inside diameter. Traditionally the tubing i.d. has been 0.5 mm. Ruzicka and Hansen developed microconduits which have tubing diameters down to 0.05 mm (1). But what happens when a process sample has particles floating around? The answer is a clogged system. The solution has been to use an in-line filter to remove the floating particles. Unfortunately, this means that the filter must be routinely changed to maintain the desired performance of the FIA system. This type of routine maintenance is not acceptable to the process chemist. An alternative is to use larger diameter tubing. It has been shown that the characteristics of an FIA system are maintained as the tubing diameter is increased. The exact upper limit is not known at this time. However, an FIA system could be made with 2.0 mm tubing with a sample

aspiration tube diameter of 0.8 mm. The aspiration tube acts as a prefilter which guarantees that the particle size in the system will be 2.5 times smaller than the manifold tubing diameter.

Pumping systems can be categorized as piston driven, peristaltic, or gas driven. The gas driven pumps have been used in process monitors. The advantages are that the system is pulseless, inexpensive, and that there are no moving parts in the pump, therefore less down time. The disadvantages are that variable flow rates will be created by the changing resistence in the reagent(s) and carrier stream storage bottles. The change in flow rates change the dispersion which in turn changes the background of the measurement. Unless this background change is well understood, the usual result will be inaccurate evaluation of the analyte concentration. The gas driven system would be hard pressed to reproducibly pump fluids in a multichannel system. Stopped-flow type measurements are difficult to perform. Techniques such as matrix modification may be possible in conventional gas driven systems.

Piston driven pumps are reasonably reliable and can handle nonaqueous solutions. The disadvantages are that they are expensive compared to the other pumping systems, pulsations created by these pumps must be dampened, stopped-flow is unlikely, and the number of moving parts is significant. The moving parts potentially lead to higher down time. The pulsation problem is the most serious. Pulsations can destroy the concentration gradient profile and create irreproducible behavior. By definition, the FIA system would no longer exist.

Peristaltic pumps appear to be the most useful for FIA. They have been used the most because they are reliable, programmable, have few moving parts, and are relatively pulseless. The preceding advantages assume, of course, that a high quality pump is used. The major advantage is that all the FIA techniques to date can be utilized in a system when peristaltic pumps are used. The disadvantages are that the pumps are moderately expensive and, more importantly, the pump tube must be replaced routinely. The routine maintenance aspect of these pumps is a serious disadvantage when unattended operation is required.

Sample introduction devices include sample injectors (both valves and syringes). For process control applications the use of syringe type injectors cannot be recommended. The valve injectors are the same as those used in low pressure HPLC. They are reliable for a period of time, then wear on the parts will begin to create irreproduciblity.

Hydrodynamic injection is a good alternative to injectors. Fig. 12.1 shows the manifold design for hydrodynamic injection.
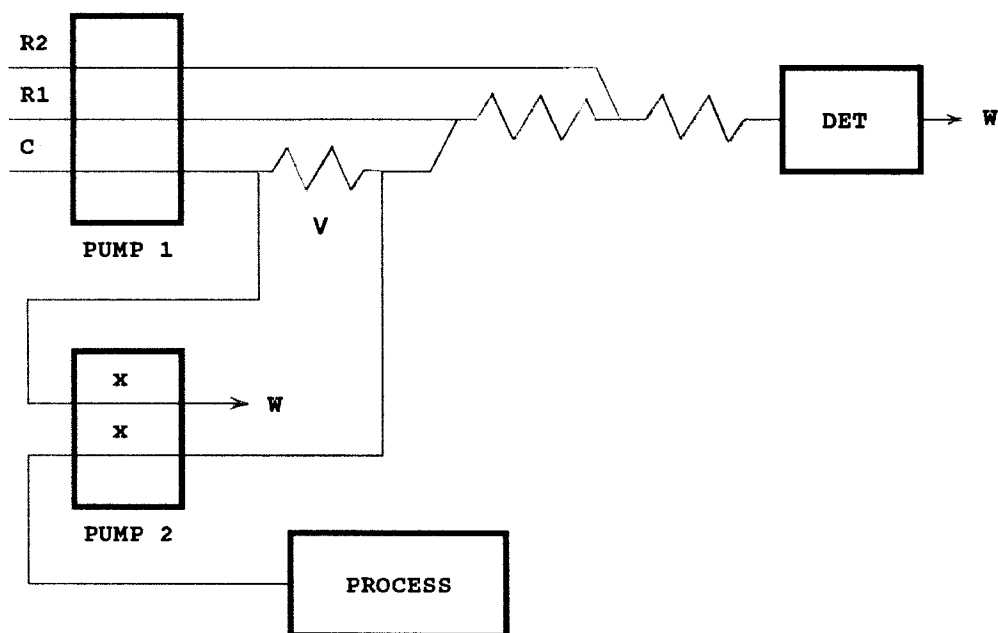
Fig. 12.1. Hydrodynamic injection manifold. C = carrier, R1, R2 = reagents, DET = detector, V = sample loop (coil), W = waste, x = flow rate for filling and for aspiration of sample.

The principle is to fill the coil V in the carrier stream with sample. This is accomplished by turning off pump 1 and starting pump 2. The coil V is filled with sample. Then pump 2 is turned off and pump 1 started. The sample in the coil is injected into the system. The reproducibility of this type of injection is comparable to the valve injectors. Note that the two flow rates used in pump 2 for filling and for aspiration of sample must be equal.

The third option to accomplish sample introduction is to pump (or aspirate) the sample and inject or merge it with the reagent(s). This reversed FIA technique is useful when sample is abundant and reagents expensive.

The last component in a process control FIA system is the detector. Essentially any existing flow through detector can be used with an FIA system. Even some detectors such as mass spectrometry have been used with FIA. As a rule, the FIA system improves the performance of the detector. In some cases such as, ion selective electrodes, or luminescence, the detector performance is greatly enhanced. For fast luminescence chemistry FIA can be used to mix and monitor the fast release of light. The readout devices usually supplied with HPLC flow through detectors will not be fast enough for FIA.

Since the FIA peak resides in the detector for only a few seconds, the software for the detector/readout device must be considerably faster for FIA than HPLC. Although the detectors can usually handle the time scale, the software usually cannot be modified by the end user.

12.3. OPERATIONAL FIA MODES IN PROCESS CONTROL

The important techniques for process monitoring in FIA fall into the category of matrix modification. In turn, this category is subdivided into three areas: analyte transplantation, dilution, and matrix matching. By far, analyte transplantation is the most important. It is used to enhance selectivity. Gas diffusion, dialysis, extraction, and columns are all analyte transplantation techniques.

Dilution is required for many process samples. One approach is to utilize the concentration gradient and use electronic dilution. This approach requires a high degree of system sophistication with respect to timing of the detector reading. The alternative is to use zone sampling, split loop injection, or a combination of the two (further details are available in Chapter 5).

The last matrix modification technique is matrix matching. For some detectors it is necessary to match the sample matrix and the matrix of the standards in order to ensure accuracy. Parameters of the sample to be considered are viscosity, ionic strength, refractive index, and pH. FIA can be easily used to produce the matrix matching.

Hopefully, the reader now has some ideas about the hardware and operational modes of FIA used in process monitoring. In the following sections some examples of process and quality control by FIA are presented. These examples are general due to the proprietary nature of exact specifications. It must be emphasized, however, that FIA is only a tool to perform chemistry.

12.4 DETERMINATION OF CYANIDE

This procedure is based on an application developed by FIAtron (FAC no. 1112). The concentration range for cyanide is 0.2 - 4.0%.

Principle

The sample, S, is injected into a carrier, C, comprising of phosphoric acid, see Fig. 12.2.
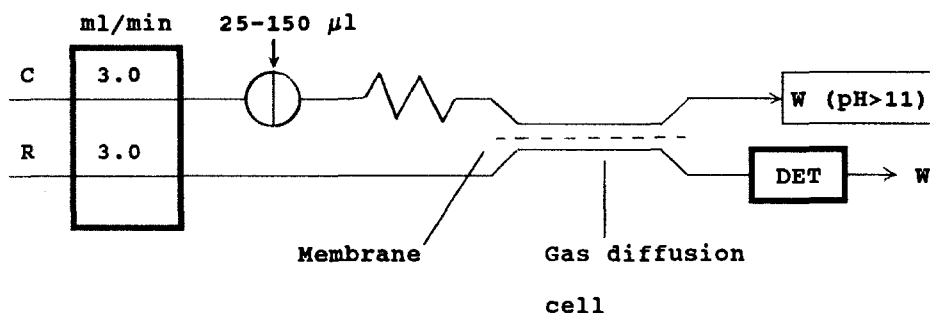
Fig. 12.2. Manifold for the determination of cyanide. The pH of the receiving solution in the waste container should be kept above 11. DET = detector system comprising a cyanide selective electrode and a reference electrode.

Hydrogen cyanide is formed which diffuses through a PTFE membrane in a gas diffusion module. Note that the carrier waste must be collected in a strongly alkaline solution (pH > 11 all the time) to neutralize the volatile hydrogen cyanide which is very toxic. The recipient stream, R, which flows through the gas diffusion module is alkaline, 0.1 M sodium hydroxide. The hydrogen cyanide which crosses the gas diffusion membrane is converted back to cyanide ions and is detected by a cyanide selective electrode.

Electrodes

The following electrodes are mounted in the ISE detection cell:
- a cyanide selective electrode
- a single junction reference electrode with KCl or a double junction reference electrode with KNO_3.

Carrier and reagent

The carrier, C, is a 1.0 M phosphoric acid solution and the reagent, R, comprises of a 0.1 M sodium hydroxide/0.1 ppm cyanide solution.

Standards

Prepare standards in the range of 0.2 - 4.0 % cyanide. Use 0.1 M sodium hydroxide as diluent.

Concluding remarks

The sample can be injected into a carrier of distilled water and merged with a reagent stream of phosphoric acid instead of the combined carrier/ reagent stream of phosphoric acid. This requires another line in the manifold in Fig. 12.2. The recipient stream, R, may contain a small amount of cyanide, 0.1 ppm, to assure a fast electrode response. Where particulate matter is present the sample must be filtered prior to the determination. An in-line filter can also be used.

Analysis of cyanide in a process stream can be performed by connecting the sample line directly to the process stream rather than interfacing with an automatic sampler. Note that the measured potential difference between the cyanide selective electrode and the reference electrode is proportional to the logarithm of the cyanide ion activity. This procedure has been used for the determination of cyanide in industrial process stream samples. The approximate composition of the process stream was: 40% acetonitrile, 10% ammonium chloride, 1 - 3% hydrogen cyanide, 47 - 50% water. The pH was 6 - 7 and the temperature 60 °C. The process stream was diluted approximately 1:50 before introduction into the FIA system. The r.s.d. was about 0.8% for a 3% cyanide solution.

12.5 NONAQUEOUS PROCESS CONTROL FIA

One of the difficulties with many first generation commercial FIA units was the fact that the manifolds and some pumps were not resistent to nonaqueous solvents. Obviously, this problem is a serious drawback in process control where organic solvents are common. To solve this problem manifolds have been made from more solvent resistent polymers or stainless steel. FIAtron has designed their process flow injection analyzer so that nonaqueous solvents can be used (APA No. 1001A), see Fig. 12.3.
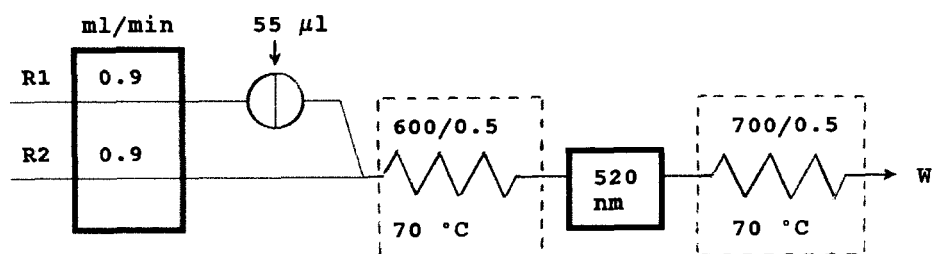
Fig. 12.3. Manifold for the determination of peroxide in isopropanol. Numbers above coils denote coil length/i.d. (cm/mm). R1 = acetic acid in isopropanol, R2 = sodium iodide in isopropanol.

One example of an organic process stream/analyte combination is peroxide in isopropyl alcohol. Peroxides have a variety of industrial uses in the chemical industry, for example, as initiators. Continuous analysis of the peroxide feedstock permits adjustments of the peroxide levels before the solution enters the process stream. The obvious result is a signficiant reduction in process costs.

The same peroxide solution is a safety problem downstream from the process. Peroxide decomposes in the process due to heating or other parameters. The oxygen released during the reaction can reach explosive levels.

Chemical reactions

The method for peroxide determination is based on the ASTM method E299-68. The peroxides oxidize I^- to I_2. Depending on the peroxide present, heating may be required to increase the reaction efficiency. Iodine present with excess of I^- forms I_3^- which absorbs visible light allowing a colorimetric determination at 520 nm. Using samples volumes in the range of 30 - 100 μl about 0.05 - 0.2 wt. % peroxide can be determined. Agreement with the ASTM method, as judged by correlation coefficient, is 0.999. A relative standard deviation of 0.5% over 15 hours is typically observed for 250 determinations.

12.6   DETERMINATION OF BASES

Bases like the hydroxide ion and the carbonate ion are usually titrated with acid using a glass electrode as a detector. When the equivalence point is approached the buffer capacity of the solution decreases and the electrode response becomes slower. The mixing efficiency must be large to guarantee stable readings throughout the whole titration. The titration procedure generates very accurate and precise results but it is tedious to apply in a process control situation where an almost instant reply is desired.

Linear buffers have been developed in order to simplify the entire titration principle and to circumvent the problems with unstable readings close to the equivalence point (2-8). The analytical procedure entails mixing of known volumes of the sample and the linear buffer. The resulting pH change is then measured. A linear relationship exists between the pH change and base concentration in the sample. The slope of the calibration curve is the inverse of the buffer capacity, ß. The buffer capacity remains constant over the entire linear range. By changing the buffer capacity the dynamic range of the method can be changed. The mixing of the linear buffer and the sample, as well as the detection, is readily performed in an FIA system (8). The linear buffer is prepared according to Table 12.1.

TABLE 12.1

Calculated amounts for preparation of 1 liter of a linear buffer for titration of bases.

Compound	ml	Conc.	g	Final conc.
Hydrochloric acid	100	1 M	-	-
Formic acid	0.85	100%	-	0.0225
Acetic acid	0.86	100%	-	0.0150
Malonic acid	-	-	1.344	0.0129
Piperazine	-	-	1.900	0.0221
Bis-Tris	-	-	2.973	0.0142
4-Methylmorpholine	2.49	99-100%	-	0.0226
N-Methyldiethanolamine	2.53	99-100%	-	0.221
Sodium chloride	-	-	58.4	1.0

Distilled water   (to a final volume of 1000 ml)

240

FIA manifold

Fig. 12.4 shows the recommended manifold.
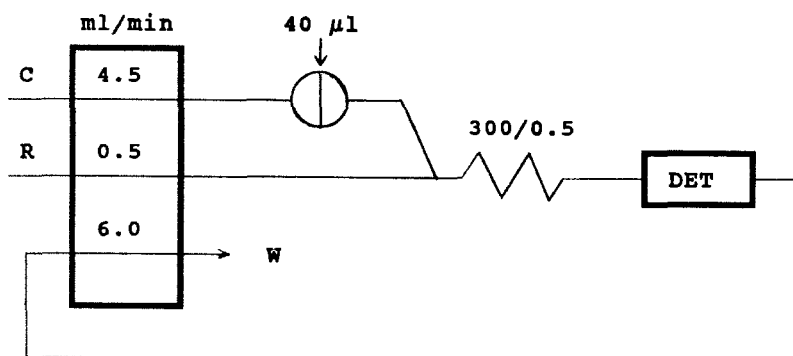
Fig. 12.4. Manifold for determination of strong bases. Numbers above coil denote coil length/i.d. (cm/mm). DET = potentiometric detector (glass electrode/reference electrode).

The sample, S, is injected into a carrier, C, comprised of distilled water. The carrier stream is merged with the linear buffer, R. A long mixing coil is required (300/0.5). A glass electrode is arranged as shown in Chapter 4, Fig. 4.5. A reference electrode of double junction type is recommended.

Concluding remarks

The sensitivity of the method depends on the ratio between C and R flows in Fig. 12.4. If the flow ratio C/R is larger than 10 a decrease in the linear range is obtained. The sample throughput is very large, above 300/h although such a high throughput is not needed in a practical situation. The working range is typically 0.01 - 0.1 M of the sodium hydroxide samples.

REFERENCES

1. J. Ruzicka and E.H. Hansen, Anal. Chim. Acta, 161 (1984) 1. [456]

2. E.B.R. Prideaux, Proc. Royal Soc. Ser. A (London), 92 (1916) 403. [-]

3. E.B.R. Prideaux and A.T. Ward, J. Chem. Soc., 125 (1924) 426. [-]

4. H.T.S. Britton and R.A. Robinson, J. Chem. Soc., 458 (1931) 1456. [-]

5. G. Johansson and W. Backen, Anal. Chim. Acta, 69 (1975) 415. [-]

6. O. Astrom, Anal. Chim. Acta, 88 (1977) 17. [-]

7. O. Astrom, Anal. Chim. Acta, 97 (1978) 259. [-]

8. O. Astrom, Anal. Chim. Acta, 105 (1979) 67. [34]

APPENDIX A

I.   USEFUL SOLUTIONS FOR DISPERSION EXPERIMENTS

Chemicals needed:
- a)   Borax, sodium tetraborate, $Na_2B_4O_7$ $10H_2O$
- b)   Bromothymol blue, 3',3''-dibromothymolsulfonphthalein, $C_{27}H_{28}Br_2O_5S$
- c)   Ethanol, 96%
- d)   Distilled water

Preparation of solutions:

1.   0.01 M Borax buffer solution, 1 liter.

Dissolve 3.814 g borax (a) in about 200 ml distilled water and add distilled water to a final volume of 1 000 ml. Degas. This solution is stable for months when stored in a normal manner.

2.   Dye stock solution, 100 ml.

Dissolve 0.400 g bromothymol blue (b) in about 25 ml ethanol (c) and make up to a final volume of 100ml with distilled water. Degas. This solution is stable for several months.

3.   Dye working solution, 200 ml.

Dilute 1 ml of the dye stock solution (2) with borax buffer (1) to a final volume of 200 ml. Degas. This solution is normally prepared freshly just before use.

II.   CONVERSION TABLE FOR COIL LENGTH/I.D./VOLUME

i.d., mm	length, cm	volume, μl	volume, μl	length, cm
0.35	10	9.6	100	104
0.5	10	19.6	100	51
0.6	10	28.3	100	35
0.7	10	38.5	100	26
0.8	10	50.3	100	20
0.9	10	63.6	100	15.7
1.0	10	78.5	100	12.7

BIBLIOGRAPHY - SUBJECT INDEX

1. SPECIES DETERMINED BY FLOW INJECTION ANALYSIS

Species	References
Acetaldehyde	884, 997, 1134
Acetaminophen	359, 432, 770
Acetazolamide	1324
Acetic acid	331
Acetoacetate	408
Acetone	308, 709
p-Acetylaminophenol	325
Acetylcholine	1355
Acetylcholinesterase	732
Acid/Base	34, 47, 161, 396, 456, 465, 483, 594, 616, 759, 782, 806, 811, 1055, 1073, 1086, 1095, 1243, 1304
Acidity	1055
Acidity constants	526, 616
Adrenaline	49, 544
Albumin	32, 37, 66, 78, 299, 393, 425, 439, 471, 636, 651, 1348
Alcohol dehydrogenase	971
Alcohols (see also Ethanol)	179, 185
Aldehydes	596
Alkaline phosphate	732
Alkalinity	517, 946
Allyl alcohol	1059
Alpha macroglobulin	299

2. AREAS OF APPLICATION

Application area	References
<u>Agriculture</u>	717, 744, 763
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Molasses	1210
Plant material	2, 3, 5, 11, 15, 20, 21, 33, 38, 40, 43, 86, 97, 113, 128, 129, 156, 159, 223, 253, 263, 334, 372, 378, 444, 453, 565, 575, 705, 717, 1070, 1137, 1339, 1361
Soil	8, 12, 38, 42, 55, 67, 130, 156, 354, 372, 515, 566, 572, 626, 963, 986, 1040, 1041, 1345
<u>Biochemistry</u>	168, 267, 282, 295, 298, 342, 420, 441, 448, 539, 553, 557, 597, 721, 741, 778, 1076, 1091, 1162, 1226, 1247, 1277, 1355
<u>Biotechnology</u>	491, 492, 493, 496, 512, 554, 559, 561, 611, 668, 776, 896, 955, 956, 1188, 1297, 1314, 1340, 1348, 1350, 1387
Fermentation	491, 493, 512, 896, 956, 1352, 1387
<u>Chemical Industry</u>	246, 271, 274, 281, 365, 392, 394, 405, 411, 421, 453, 627, 637, 684, 791, 847, 858, 955, 988, 1008, 1015, 1086, 1193, 1266, 1340
Alloys	157, 184, 199, 879, 984, 1364, 1377
Brine	1033, 1265
Coal fly ash	565
Ethanol	647, 1196
Gasoline	637, 762

Water (continued)	1123, 1131, 1136, 1139, 1145, 1148, 1150, 1161, 1169, 1184, 1207, 1208, 1211, 1219, 1228, 1229, 1230, 1232, 1235, 1239, 1242, 1268, 1279, 1283, 1291, 1294, 1298, 1299, 1300, 1301, 1302, 1304, 1305, 1306, 1307, 1309, 1312, 1317, 1319, 1320, 1321, 1322, 1326, 1329, 1331, 1338, 1339, 1345, 1360, 1392
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Beer	161, 942, 1275
Beverages	185, 308, 349, 376, 424, 460, 962, 1311
Butter	640
Cereals	672
Coffee	684
Flour	943
Fruit juice	848, 1210, 1244
Fruits	460, 856
Jam	848
Meat	744, 1190, 1208, 1238
Milk	44, 308, 621, 780, 785, 898, 916
Rice	684
Rice flour	565
Soft drinks	672, 1244
Sugar cane	1210, 1362
Tea	718
Wheat flour	565
Wine	754, 855, 884, 997, 1006, 1196, 1382

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AAS (Atomic Absorption Spectroscopy)	33, 50, 53, 85, 90, 98, 113, 140, 151, 173, 175, 178, 188, 190, 233, 240, 274, 286, 302, 303, 304, 306, 317, 330, 347, 352, 371, 381, 383, 389, 397, 406, 410, 417, 423, 427, 428, 435, 444, 447, 450, 454, 476, 477, 482, 497, 498, 523, 564, 565, 566, 576, 599, 601, 603, 618, 634, 635, 647, 658, 669, 671, 687, 688, 689, 690, 693, 736, 743, 744, 745, 760, 762, 764, 793, 818, 822, 827, 833, 837, 850, 851, 853, 869, 880, 895, 907, 918, 920, 976, 983, 1014, 1016, 1031, 1032, 1033, 1084, 1085, 1094, 1096, 1123, 1154, 1178, 1197, 1230, 1255, 1339, 1345, 1346, 1388, 1389, 1390
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4. ELECTROCHEMICAL TECHNIQUES

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6. GRADIENT AND MATRIX MODIFICATION TECHNIQUES

Technique/Method	References
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7. MISCELLANEOUS KEYWORDS

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BIBLIOGRAPHY - REFERENCES

1.   J. Ruzicka and E.H. Hansen, Flow Injection Analysis. Part I. A New Concept of Fast Continuous Flow Analysis, Anal. Chim. Acta, 78 (1975) 145-157.

2.   J. Ruzicka and J.W.B. Stewart, Flow Injection Analysis. Part II. Ultrafast Determination of Phosphorous in Plant Material by Continuous Flow Spectrophotometry, Anal. Chim. Acta, 79 (1975) 79-91.

3.   J.W.B. Stewart, J. Ruzicka, H. Bergamin Filho and E.A.G. Zagatto, Flow Injection Analysis. Part III. Comparison of Continuous Flow Spectrophotometry and Potentiometry for Rapid Determination of the Total Nitrogen Content in Plant Digests, Anal. Chim. Acta, 81 (1976) 371-386.

4.   J. Ruzicka, J.W.B. Stewart and E.A.G. Zagatto, Flow Injection Analysis. Part IV. Stream Sample Splitting and Its Application to the Continuous Spectrophotometric Determination of Chloride in Brackish Waters, Anal. Chim. Acta, 81 (1976) 387-396.

5.   J.W.B. Stewart and J. Ruzicka, Flow Injection Analysis. Part V. Simultaneous Determination of Nitrogen and Phosphorous in Acid Digests of Plant Material with a Single Spectrophotometer, Anal. Chim. Acta, 82 (1976) 137-144.

6.   J. Ruzicka and E.H. Hansen, Flow Injection Analysis. Part VI. The Determination of Phosphate and Chloride in Blood Serum by Dialysis and Sample Dilution, Anal. Chim. Acta, 87 (1976) 353-363.

7.   D. Betteridge and J. Ruzicka, The Determination of Glycerol in Water by Flow Injection Analysis - A Novel Way of Measuring Viscosity, Talanta, 23 (1976) 409-410.

8.   J. Ruzicka, E.H. Hansen and E.A.G. Zagatto, Flow Injection Analysis. Part VII. Use of Ion-Selective Electrodes for Rapid Analysis of Soil Extracts and Blood Serum. Determination of Potassium, Sodium and Nitrate, Anal. Chim. Acta, 88 (1977) 1-16.

9.   E.H. Hansen, J. Ruzicka and B. Rietz, Flow Injection Analysis. Part VIII. Determination of Glucose in Blood Serum with Glucose Dehydrogenase, Anal. Chim. Acta, 89 (1977) 241-254.

10.   J. Ruzicka, E.H. Hansen and H. Mosbaek, Flow Injection Analysis, Part IX. A New Approach to Continuous Flow Titrations, Anal. Chim. Acta, 92 (1977) 235-249.

11.   F.J. Krug, H. Bergamin Filho, E.A.G. Zagatto and S.S. Jorgensen, Rapid Determination of Sulphate in Natural Waters and Plant Digests by Continuous Flow Injection Turbidimetry, Analyst, 102 (1977) 503-508.

12.   E.H. Hansen, A.K. Ghose and J. Ruzicka, Flow Injection Analysis of Nitrate in Environmental Samples Using an Ion-Selective Electrode, Analyst, 102 (1977) 705-713.

13.   E.H. Hansen, F.J. Krug, A.K. Ghose and J. Ruzicka, Rapid Determination of N, P, and K in Fertilizers by Flow Injection Analysis, Analyst, 102 (1977) 714-722.

274

14. J. Ruzicka, E.H. Hansen, H. Mosbaek and F.J. Krug, Pumping Pressure and Reagent Consumption in Flow Injection Analysis, Anal. Chem., 49 (1977) 1858-1862.

15. L. Sodek, J. Ruzicka and J.W.B. Stewart, Rapid Determination of Protein in Plant Material by Flow Injection Spectrophotometry with Trinitobenzenesulfonic Acid, Anal. Chim. Acta, 97 (1978) 327-333.

16. H. Bergamin Filho, B.F. Reis and E.A.G. Zagatto, A New Device for Improving Sensitivity and Stabilization in Flow Injection Analysis, Anal. Chim. Acta, 97 (1978) 427-431.

17. S. Thelander and B. Karlberg, Extraction Based on the Flow Injection Principle. Part I. Description of the Extraction System, Anal. Chim. Acta, 98 (1978) 1-7.

18. J. Ruzicka and E.H. Hansen, Flow Injection Analysis. Part X. Theory, Techniques and Trends, Anal. Chim. Acta, 99 (1978) 37-76.

19. E.H. Hansen, J. Ruzicka and A.K. Ghose, Flow Injection Analysis of Calcium in Serum, Water and Waste Water by Spectrophotometry and by Ion-Selective Electrode, Anal. Chim. Acta, 100 (1978) 151-165.

20. H. Bergamin Filho, J.X. Medeiros, B.F. Reis and E.A.G. Zagatto, Solvent Extraction in Continuous Flow Analysis. Determination of Molybdenum in Plant Material, Anal. Chim. Acta, 101 (1978) 9-16.

21. H. Bergamin Filho, E.A.G. Zagatto, F.J. Krug and B.F. Reis, Merging Zones in Flow Injection Analysis. Part I. Double Proportional Injector and Reagent Consumption, Anal. Chim. Acta, 101 (1978) 17-23.

22. W.E. Van der Linden and R. Oostervink, Construction and Behaviour of a Micro Flow-through Copper(II)-Selective Electrode, Anal. Chim. Acta, 101 (1978) 419-422.

23. W.D Basson and J.F. Van Staden, Use of Non-Segmented High-Speed Continuous Flow Analysis for the Determination of Calcium in Animal Feeds, Analyst, 103 (1978) 296-299.

24. D. Betteridge, E.L. Dagless, B. Fields and N.F. Graves, A Highly Sensitive Flow-through-Phototranducer for Unsegmented CFA Demonstrating High-Speed Spectrophotometry at the ppb Level and a New Method of Refractometric Determinations, Analyst, 103 (1978) 897-908.

25. W.D. Basson and J.F. Van Staden, Low-Level Determination of Hydrazine in Boiler Feed Water with an Unsegmented High-Speed Continuous Flow System, Analyst, 103 (1978) 998-1001.

26. S. Thelander and B. Karlberg, Determination of Readily Oxidised Compounds by Flow Injection Analysis and Redox Potential Detection, Analyst, 103 (1978) 1154-1159.

27. K. Kina, K. Shiraishi and N. Isibashi, Ultramicro Solvent Extraction and Fluorimetry Based on the Flow Injection Method, Talanta, 25 (1978) 295-297.

28. D. Betteridge and B. Fields, Constructions of pH Gradients in Flow Injection Analysis and Their Potential Use for Multielement Analysis in a Single Sample Bolus, Anal. Chem., 50 (1978) 654-656.

29.   D. Betteridge, Flow Injection Analysis, Anal. Chem., 50 (1978) 832A-842A.

30.   J. Slanina, F. Bakker, A.G.M. Bruijn-Hes and J.J. Mols, Fast Determination of Nitrate in Small Samples of Rain and Surface Waters by Means of UV Spectrophotometry and Flow Injection Analysis, Fresenius' Z. Anal. Chem., 289 (1978) 38-40.

31.   B. Karlberg, P.A. Johansson and S. Thelander, Extraction Based on the Flow Injection Principle. Part II. Determination of Codeine as the Picrate Ion-Pair in Acetylsalicylic Acid Tablets, Anal. Chim. Acta, 104 (1979) 21-28.

32.   J. Mindegaard, Flow Multi-Injection Analysis - A System for the Analysis of Highly Concentrated Samples without Prior Dilution, Anal. Chim. Acta, 104 (1979) 185-189.

33.   E.A.G. Zagatto, F.J. Krug, H. Bergamin Filho, S.S. Jorgensen and B.F. Reis, Merging Zones in FIA. Part 2. Determination of Calcium, Magnesium and Potassium in Plant Material by Continuous Flow Injection Atomic Absorption and Flame Emission Spectrometry, Anal. Chim. Acta, 104 (1979), 279-284.

34.   O. Astrom, Single-Point Titration. Part 4. Determination of Acids and Bases with Flow Injection Analysis, Anal. Chim. Acta, 105 (1979) 67-75.

35.   J.H. Dahl, D. Espersen and A. Jensen, Differential Kinetic Analysis and Flow Injection Analysis. Part I. The Trans-1,2-diaminocyclohexane-tetraacetate Complexes of Magnesium and Strontium, Anal. Chim. Acta, 105 (1979) 327-333.

36.   J. Ruzicka and E.H. Hansen, Stopped-Flow and Merging Zones - A New Approach to Enzymatic Assays by Flow Injection Analysis. Determination of Glucose in Serum, Anal. Chim. Acta, 106 (1979) 207-224.

37.   J.I. Braithwaite and J.N. Miller, Flow Injection Analysis with a Fluorimetric Detector for Determinations of Glycine and Albumin, Anal. Chim. Acta, 106 (1979) 395-399.

38.   B.F. Reis, H. Bergamin Filho, E.A.G. Zagatto and F.J. Krug, Merging Zones in Flow Injection Analysis. Part 3. Spectrophotometric Determination of Aluminium in Plant and Soil Materials with Sequential Addition of Pulsed Reagents, Anal. Chim. Acta, 107 (1979) 309-317.

39.   D. Espersen and A. Jensen, Differential Kinetic Analysis and Flow Injection Analysis. Part II. The (2.2.1) Cryptates of Magnesium and Calcium, Anal. Chim. Acta, 108 (1979) 241-247.

40.   E.A.G. Zagatto, B.F. Reis, H. Bergamin Filho and F.J. Krug, Isothermal Distillation in Flow Injection Analysis. Determination of Total Nitrogen in Plant Materials, Anal. Chim. Acta, 109 (1979) 45-54.

41.   L. Andersson, Simultaneous Spectrophotometric Determination of Nitrite and Nitrate by Flow Injection Analysis, Anal. Chim. Acta, 110 (1979) 123-128.

42.   F.J. Krug, J. Ruzicka and E.H. Hansen, Determination of Ammonia in Low Concentrations with Nessler's Reagent by Flow Injection Analysis, Analyst, 104 (1979) 47-54.

43.   M.F. Gine, E.A.G. Zagatto and H. Bergamin Filho, Semiautomatic Determination of Manganese in Natural Waters and Plants Digests by Flow Injection Analysis, Analyst, 104 (1979) 371-375.

44.   W.D. Basson and J.F. Van Staden, Direct Determination of Calcium in Milk on a Non-Segmented Continuous Flow System, Analyst, 104 (1979) 419-424.

45.   H. Baadenhuijen and H.E.H. Seuren-Jacobs, Determination of Total CO_2 in Plasma by Automated Flow Injection Analysis, Clin. Chem., 28 (1979) 443-445.

46.   G. Rule and W.R. Seitz, Flow Injection Analysis with Chemiluminescence Detection, Clin. Chem., 25 (1979) 1635-1638.

47.   E.H. Hansen and J. Ruzicka, The Priciples of Flow Injection Analysis as Demonstrated by Three Lab. Exercises, J. Chem. Ed., 56 (1979) 677-680.

48.   J. Ruzicka, E.H. Hansen, A.K. Ghose and H.A. Mottola, Enzymatic Determination of Urea in Serum based on pH Measurement with the Flow Injection Method, Anal. Chem., 51 (1979) 199-203.

49.   A.N. Strohl and D.J. Curran, Flow Injection Analysis with Reticulated Vitreous Carbon Flow-through Electrodes, Anal. Chem., 51 (1979) 1045-1049.

50.   W.R. Wolf and K.K. Stewart, Automated Multiple Flow Injection Analysis for Flame Atomic Absorption Spectrometry, Anal. Chem., 51 (1979) 1201-1205.

51.   J. Kawase, A. Nakae and M. Yamanaka, Determination of Anionic Surfactants by Flow Injection Analysis Based on Ion-Pair Extraction, Anal. Chem., 51 (1979) 1640-1643.

52.   J. Ruzicka and E.H. Hansen, Flow Injection Analysis, Chem. Tech., (1979) 756-764.

53.   N. Yoza, Y. Aoyagi, S. Ohashi and A. Tateda, Flow Injection System for Atomic Absorption Spectrometry, Anal. Chim. Acta, 111 (1979) 163-167.

54.   H.K. Chan and A.G. Fogg, Flow Injection Determination of Meptazinol with Electrochemical Detection, Anal. Chim. Acta, 111 (1979) 281-285.

55.   S.S. Jorgensen and M.A.B. Regitano, Rapid Determination of Chromium(VI) by Flow Injection Analysis, Analyst, 105 (1980) 292-294.

56.   L.R. Snyder, Continuous Flow Analysis; Present and Future, Anal. Chim. Acta, 114 (1980) 3-18.

57.   J. Ruzicka and E.H. Hansen, Flow Injection Analysis. Principles, Application and Trends, Anal. Chim. Acta, 114 (1980) 19-44.

58.   H. Poppe, Characterization and Design of Liquid Phase Flow-through Detector Systems, Anal. Chim. Acta, 114 (1980) 59-70.

59.   R. Tijssen, Axial Dispersion and Flow Phenomena in Helically Coiled Tubular Reactors for Flow Analysis and Chromatography, Anal. Chim. Acta, 114 (1980) 71-89.

60.   J.H.M. Van den Berg, R.S. Deelder and H.G.M. Egberink, Dispersion Phenomena in Reactors for Flow Analysis, Anal. Chim. Acta, 114 (1980) 91-104.

61.   J.M. Reijn, W.E. Van der Linden and H. Poppe, Some Theoretical Aspects of Flow Injection Analysis, Anal. Chim. Acta, 114 (1980) 105-118.

62. K.K. Stewart, J.F. Brown and B.M. Golden, A Microprocessor Control System for Automated Multiple Flow Injection Analysis, Anal. Chim. Acta, 114 (1980) 119-127.

63. B. Karlberg and S. Thelander, Extraction Based on the Flow Injection Principle. Part 3. Fluorimetric Determination of Vitamin B1 (Thiamine) by the Thiochrome Method, Anal. Chim. Acta, 114 (1980) 129-136.

64. J.B. Landis, Rapid Determination of Corticosteroids in Pharmaceuticals by Flow Injection Analysis, Anal. Chim. Acta, 114 (1980) 155-163.

65. A. Ramsing, J. Ruzicka and E.H. Hansen, A New Approach to Enzymatic Assay Based on Flow Injection Spectrophotometry with Acid-Base Indicators, Anal. Chim. Acta, 114 (1980) 165-181.

66. C.S. Lim, J.N. Miller and J.W. Bridges, Automation of an Energy-Transfer Immunoassay by Using Stopped Flow Injection Analysis with Merging Zones, Anal. Chim. Acta, 114 (1980) 183-189.

67. M.F. Gine, H. Bergamin Filho, E.A.G. Zagatto and B.F. Reis, Simultaneous Determination of Nitrate and Nitrite by Flow Injection Analysis, Anal. Chim. Acta, 114 (1980) 191-197.

68. I. Kagevall, O. Astrom and A. Cedergren, Determination of Water by Flow Injection Analysis, Anal. Chim. Acta, 114 (1980) 199-208.

69. J.L. Burguera, A. Townshend and S. Greenfield, Flow Injection Analysis for Monitoring Chemiluminescent Reactions, Anal. Chim. Acta, 114 (1980) 209-214.

70. P.A. Johansson, B. Karlberg and S. Thelander, Extraction Based on the Flow Injection Principle. Part 4. Determination of Extraction Constants, Anal. Chim. Acta, 114 (1980) 215-226.

71. H. Kagenow and A. Jensen, Differential Kinetics Analysis and Flow Injection Analysis. Part 3. The (2.2.2) Cryptates of Magnesium, Calcium and Strontium, Anal. Chim. Acta, 114 (1980) 227-234.

72. J.W. Dieker and W.E. Van der Linden, Determination of Iron(II) and Iron(III) by Flow Injection and Amperometric Detection with a Glassy Carbon Electrode, Anal. Chim. Acta, 114 (1980) 267-274.

73. S. Baban, D. Beetlestone, D. Betteridge and P. Sweet, The Determination of Sulphate by Flow Injection Analysis with Exploitation of pH Gradients and EDTA, Anal. Chim. Acta, 114 (1980) 319-323.

74. N. Ishibishi, K. Kina and Y. Goto, Selective Determination of Gallium by Flow Injection, Anal. Chim. Acta, 114 (1980) 325-328.

75. Y. Hirai, N. Yoza and S. Ohashi, Flow Injection Analysis of Inorganic Polyphosphates, Anal. Chim. Acta, 115 (1980) 269-277.

76. O. Klinghoffer, J. Ruzicka and E.H. Hansen, Flow Injection Analysis of Traces of Cadmium and Lead by Solvent Extraction by Dithizone, Talanta, 27 (1980) 169-175.

278

77.   J. Slanina, F. Bakker, A. Bruyn-Hes and J.J. Mols, A Computer Controlled Multichannel Continuous Flow Analysis System Applied to the Measurement of Nitrate, Chloride, and Ammonium Ions in Small Samples of Rain Water, Anal. Chim. Acta, 113 (1980) 331-342.

78.   B.W. Renoe, K.K. Stewart, G.R. Beecher, M.R. Wills and J. Savory, Automated Multiple Flow Injection Analysis in Clinical Chemistry: Determination of Albumin with Bromcresol Green, Clin. Chem., 26 (1980) 331-334.

79.   D. Espersen, H. Kagenow and A. Jensen, Flow Injection Analysis and Differential Kinetic Analysis, Arch. Pharm. Chem. Sci. Ed., 8 (1980) 53-62.

80.   B. Fields, Studies in Flow Injection Analysis, Proc. Anal. Div. Chem. Soc., 16 (1979) 4-7.

81.   J.L. Burguera and A. Townshend, Monitoring Chemiluminescent Reactions by Flow Injection Analysis, Proc. Anal. Div. Chem. Soc., 16 (1979) 263-264.

82.   J.A. Lown, R Koile and D.C. Johnson, Amperometric Flow-through Wire Detector: A Practical Design with High Sensitivity, Anal. Chim. Acta, 116 (1980) 33-39.

83.   J.A. Lown and D.C. Johnson, Anodic Detection of Arsenic(III) in a Flow-through Platinum Electrode for Flow Injection Analysis, Anal. Chim. Acta, 116 (1980) 41-51.

84.   Y. Hirai, N. Yoza and S. Ohashi, Flow Injection Analysis of Inorganic Ortho- and Polyphosphates Using Ascorbic Acid as a Reductant of Molybdophosphate, Chem. Lett., 5 (1980) 499-502.

85.   B.W. Renoe and A. Obrien, Calcium by Flow Injection Atomic Absorption, Clin. Chem., 26 (1980) 1021-1022.

86.   N.W. Holt, Flow Injection Analysis - Adaption to a Small Laboratory, Can. J. Plant. Sci., 60 (1980) 767-769.

87.   H. Bergamin Filho, B.F. Reis, A.O. Jacintho and E.A.G. Zagatto, Ion Exchange in Flow Injection Analysis. Determination of Ammonium Ions at the μg/l Level in Natural Waters with Pulsed Nessler Reagent, Anal. Chim. Acta, 117 (1980) 81-89.

88.   J. Slanina, W.A. Lingerak and F. Bakker, The Use of Ion-Selective Electrodes in Manual and Computer Contolled Flow Injection Systems, Anal. Chim. Acta, 117 (1980) 91-98.

89.   A. Ramsing, J. Janata, J. Ruzicka and M. Levy, Miniaturization in Analytical Chemistry - A Combination of Flow Injection Analysis and Ion-Sensitive Field-Effect Transistors for Determination of pH, and Potassium and Calcium, Anal. Chim. Acta, 118 (1980) 45-52.

90.   W.D. Basson and J.F. Van Staden, Simultaneous Determination of Sodium Potassium, Magnesium and Calcium in Surface, Ground, and Domestic Water by Flow Injection Analysis, Fresenius' Z. Anal. Chem., 302 (1980) 370-374.

91.   P. Maitoza and D.C. Johnson, Detection of Metal Ions without Interference from Dissolved Oxygen by Reverse Pulse Amperometry in Flow Injection Systems and Liquid Chromatography, Anal. Chim. Acta, 118 (1980) 233-241.

92.   L. Nord and B. Karlberg, Extraction Based on the Flow Injection Principle. Part 5. Assessment with a Membrane Phase Separator for Different Organic Solvents, Anal. Chim. Acta, 118 (1980) 285-292.

93.   T. Korenaga, A Flow Injection Analyzer for Chemical Oxygen-Demand Using Potassium Permanganate, Chem. Biomed. Environ. Instrum., 10 (1980) 273-287.

94.   C.E. Shideler, B.W. Renoe, J. Crump, M.R. Wills, J. Savory and K.K. Stewart, Automated Multiple Flow-Injection Analysis in Clinical Chemistry: Determination of Total Protein with Biuret Reagent, Clin. Chem., 26 (1980) 1011-1013.

95.   B.J. Compton, J.R. Weber and W.C. Purdy, Stop-Flow Analysis: An Aid in the Diagnosis and Optimization of Continuous-Flow Systems, Anal. Lett. B, 13 (1980) 861-870.

96.   T. Korenaga, Flow Injection Analysis Using Potassium Permanganate: An Approach for Measuring Chemical Oxygen Demand in Organic Waste and Waters, Anal. Lett. A, 13 (1980) 1001-1011.

97.   B.F. Reis, E.A.G. Zagatto, A.O. Jacintho, F.J. Krug and H. Bergamin Filho, Merging Zones in Flow Injection Analysis. Part 4. Simultaneous Spectrophotometric Determination of Total Nitrogen and Phosphorous in Plant Materials, Anal. Chim. Acta, 119 (1980) 305-311.

98.   K. Fukamachi and N. Ishibashi, Flow Injection. Atomic Absorption Spectrometry with Organic Solvents, Anal. Chim. Acta, 119 (1980) 383-387.

99.   T. Yumane and T. Fukasawa, Flow Injection Determination of Trace Vanadium with Catalytic Photometric Detection, Anal.Chim. Acta, 119 (1980) 389-392.

100.   S.M. Ramasamy, M.S.A. Jabbar and H.A. Mottola, Flow Injection Analysis Based on Two Consecutive Reactions at a Gas-Solid Interface for Determination of Bromine and Chlorine, Anal. Chem., 52 (1980) 2062-2066.

101.   D. Betteridge, Analytical Chemistry - the Numbers Game, Chem. Brit., 16 (1980) 646-650.

102.   T.N. Morrison, K.G. Schick and C.O. Huber, Determination of Ethanol by Air-Stream Separation with Flow Injection and Electrochemical Detection at a Nickel Oxide Electrode, Anal. Chim. Acta, 120 (1980) 75-80.

103.   E.A.G. Zagatto, A.O. Jacintho, J. Mortatti and H. Bergamin Filho, An Improved Flow Injection Determination of Nitrite in Waters by Using Intermittent Flows, Anal. Chim. Acta, 120 (1980) 339-403.

104.   N. Yoza, Y. Kurokawa, Y. Hirai and S. Ohashi, Flow Injection Determination of Polyphosphates Based on Colored Metal Complexes of Xylenol Orange and Methylthymol Blue, Anal. Chim. Acta, 121 (1980) 281-287.

105.   E.J. Duffield, G.J. Moody and J.D.R. Thomas, Development of Ion-Selective Electrodes and Flow Injection Analysis for Sulphides and Thiols, Proc. Anal. Div. Chem. Soc., 17 (1980) 533-535.

106.   S. Baban, Recent Development in Flow Injection Analysis: Determination of Bismuth, Thorium, and Copper with Pyrocatechol Violet by Exploitation of pH, Proc. Anal. Div. Chem. Soc., 17 (1980) 535.

280

107.   J. Kawase, Automated Determination of Cationic Surfactants by Flow Injection Analysis Based on Ion-Pair Extraction, Anal. Chem., 52 (1980) 2124-2127.

108.   D. Betteridge, E.L. Dagless, B. Fields, P. Sweet and D.R. Deans, Flow Injection Techniques. Analytical Chemistry at the Interface, Proc. Anal. Div. Chem. Soc., 18 (1981) 26-31.

109.   C.B. Ranger, Flow Injection Analysis. Principles, Techniques, Applications, and Designs, Anal. Chem., 53 (1981) 20A-30A.

110.   J.T. Vanderslice, K.K. Stewart, A.G. Rosenfeld and D.J. Higgs, Laminar Dispersion in Flow-Injection Analysis, Talanta, 28 (1981) 11-18.

111.   V. Baltensperger and R. Eggli, Characteristics of an Amperometric Flow-through Detector with a Renewable Stationary Mercury Electrode, Anal. Chim. Acta, 123 (1981) 107-114.

112.   B. Persson and L. Rosen, Flow Injection Determination of Isosorbide Dinitrate with Polarographic Detection, Anal. Chim. Acta, 123 (1981) 115-123.

113.   B.F. Reis, A.O. Jacintho, J. Mortatti, F.J. Krug, E.A.G. Zagatto, H. Bergamin Filho and L.C.R. Pessenda, Zone-Sampling Processes in Flow Injection Analysis, Anal. Chim. Acta, 123 (1981) 221-228.

114.   J.M. Reijn, W.E. Van der Linden and H. Poppe, Dispersion in Open Tubes Packed with Large Glass Beads. The Single Bead String Reactor, Anal. Chim. Acta, 123 (1981) 229-237.

115.   T.R. Williams, S.W. McElvany and E.C. Ighodalo, Determination of Sulfur Dioxide in Solutions by Pyridinium Bromide Perbromide and Tititrimetric and Flow Injection, Anal. Chim. Acta, 123 (1981) 351-354.

116.   W.J. Blaedel and J. Wang, Symmetrical Two-Electrode Pulsed-Flow Detector for Liquid Chromatography, Anal. Chem., 53 (1981) 78-80.

117.   H.L. Pardue and B. Fields, Kinetic Treatment of Unsegmented Flow Systems. Part 1. Subjective and Semiquantitative Evaluations of Flow Injection Systems with Gradient Chamber, Anal. Chim. Acta, 124 (1981) 39-63.

118.   H.L. Pardue and B. Fields, Kinetic Treatment of Unsegmented Flow Systems. Part 2. Detailed Treatment of Flow Injection Systems with Gradient Chamber, Anal. Chim. Acta, 124 (1981) 65-79.

119.   J. Ruzicka and E.H. Hansen, Flow Injection Analysis. Chemical Analysis Series, Vol 62, John Wiley and Sons, New York, 1981.

120.   P.L. Meschi and D.C. Johnson, The Amperometric Response of Tubular Electrodes Applied to Flow Injection Determinations, Anal. Chim. Acta, 124 (1981) 303-314.

121.   P.L. Meschi, D.C. Johnson and G.R. Luecke, The Coulometric Response of Tubular Electrodes Applied to Flow Injection Determinations, Anal. Chim. Acta, 124 (1981) 315-320.

122.   B.C. Madsen, Utilization of Flow Injection with Hydrazine Reduction and Photometric Detection for the Determination of Nitrate in Rain-Water, Anal. Chim. Acta, 124 (1981) 437-441.

123.   K.K. Stewart and A.C. Rosenfeld, Automated Titrations: The Use of Automated Multiple Flow Injection Analysis for the Titration of Discrete Samples, J. Autom. Chem., 3 (1981) 30-32.

124.   S.J. Lyle and M.I. Saleh, Observations on a Dropping-Mercury Electrochemical Detector for Flow Injection Analysis and HPLC, Talanta, 28 (1981) 251-254.

125.   J.F. Van Staden and W.D. Basson, Automated Flow-Injection Analysis of Urinary Inorganic Sulfates, Lab. Pract., 29 (1980) 1279-1280.

126.   Y. Hirai, N. Yoza and S. Ohashi, Flow Injection System as a Post-Column Reaction Detector for High-Performance Liquid Chromatography of Phosphinate, Phosphonate, and Orthophosphate, Chromatogr. 206 (1981) 501-509.

127.   G. Ham, Refractive Index Effect in Flow Injection Analysis, Anal. Proc. Div. Chem. Soc., 18 (1981) 69-70.

128.   E.A.G. Zagatto, A.O. Jacintho, L.C.R. Pressenda, J.F. Krug, B.F. Reis and H. Bergamin Filho, Merging Zones in Flow Injection Analysis. Part 5. Simultaneous Determination of Aluminium and Iron in Plant Digest by a Zone-Sampling Approach, Anal. Chim. Acta, 125 (1981) 37-43.

129.   F.J. Krug, J. Mortatti, L.C.R. Pessenda, E.A.G. Zagatto and H. Bergamin Filho, Flow Injection Spectrophotometric Determination of Boron in Plant Material with Azomethine-H, Anal. Chim. Acta, 125 (1981) 29-35.

130.   E.B. Schalscha, T. Schirado and I. Vergara, Flow Injection Analysis of Nitrate in Soil Extracts - Evaluation of a Nitrate-Selective Electrode Method, Soil Sci. Soc. Am. J., 45 (1981) 446-448.

131.   W.D. Basson and J.F. Van Staden, Simultaneous Determination of Chloride and Sulfate in Natural Waters by Flow-Injection Analysis, Water Res., 15 (1981) 333-335.

132.   J.L. Burguera, M. Burguera and A. Townshend, Determination of Zinc and Cadmium by Flow Injection Analysis and Chemiluminescence, Anal. Chim. Acta, 127 (1981) 199-201.

133.   M.E. Meyerhoff and Y.M. Fraticelli, Flow Injection Determination of Ammonia-N Using Polymer Membrane Electrode-Based Gas Sensing System, Anal. Lett., 14 (1981) 415-432.

134.   S.M. Ramasamy and H.A. Mottola, Flow Injection (Closed-Loop Configuration) Catalytic Determination of Copper in Human Blood Serum, Anal. Chim. Acta, 127 (1981) 39-46.

135.   J.M. Reijn, W.E. Van der Linden and H. Poppe, Transport Phenomena in Flow Injection Analysis without Chemical Reaction, Anal. Chim. Acta, 126 (1981) 1-13.

136.   D.J. Leggett, N.H. Chen and D.S. Mahadevappa, Flow Injection Method for Sulfide Determination by the Methylene Blue Method, Anal. Chim. Acta, 128 (1981) 163-167.

137.   T. Korenaga and H. Ikatsu, Continuous Flow Injection Analysis of Aqueous Environmental Samples for Chemical Oxygen Demand, Analyst, 106 (1981) 653-662.

282

138.   C.C. Painton and H.A. Mottola, Chemical Kinetic Contributions to Practical Dispersion in Flow Injection Analysis, Anal. Chem., 53 (1981) 1713-1715.

139.   A. Ramsing, J. Ruzicka and E.H. Hansen, The Principles and Theory of High-Speed Titrations by Flow Injection Analysis, Anal. Chim. Acta, 129 (1981) 1-17.

140.   B.D. Mindel and B. Karlberg, A Sample Pretreatment System for Atomic Absorption Using Flow Injection Analysis, Lab. Pract., 30 (1981) 719-723.

141.   T. Imasaka, T. Harada and N. Ishibashi, Fluorimetric Determination of Gallium with Lumogallion by Flow Injection Analysis Based on Solvent Extraction, Anal. Chim. Acta, 129 (1981) 195-203.

142.   B.C. Madsen and J.R. Murphy, Flow Injection and Photometric Determination of Sulfate in Rainwater with Methylthymol Blue, Anal. Chem., 53 (1981) 1924-1926.

143.   T.R. Lindstrom and D.C. Johnson, Evaluation of n-app for the Underpotential Deposition of Mercury on Gold by Flow Injection Coulometry, Anal. Chem., 53 (1981) 1855-1857.

144.   S. Greenfield, FIA (Flow Injection Analysis) Weds ICP (Inductively Coupled Plasma) - a Marriage of Convenience, Ind. Res. Dev., 23 (1981) 140-145.

145.   A. Deratani and B. Sebille, Metal Ion Exchange with a Thiol Hydrophilic Resin, Anal. Chem., 53 (1981) 1742-1746.

146.   W.D. Basson, J.F. Van Staden and P.M. Cattin, Determination of Phosphorous as Molybdovanadosphosporic Acid in Phosphate Rock with a Flow-Injection Procedure, Fresenius' Z. Anal. Chem., 307 (1981) 373-377.

147.   T. Yamane, Flow Injection Determination of Traces of Cobalt by Catalysis of the SPADNS-Hydrogen Peroxide Reaction with Spectrophotometric Detection, Anal. Chim. Acta, 130 (1981) 65-72.

148.   H.W. Van Rooijen and H. Poppe, An Electrochemical Reactivation Method for Solid Electrodes Used in Electrochemical Detectors for High-Performance Liquid Chromatography and Flow Injection Analysis, Anal. Chim. Acta, 130 (1981) 9-22.

149.   J.N. Miller, Luminescence Detection in Flow Injection Analysis, Anal. Div. Proc. Chem. Soc., 18 (1981) 264-267.

150.   T.A. Kelly and G.D. Christian, Fluorometer for Flow Injection Analysis with Application to Oxidase Enzyme Dependent Reactions, Anal. Chem., 53 (1981) 2110-2114.

151.   B.W. Roe, C.E. Shideler and J. Savory, Use of a Flow-Injection Sample Manipulator as an Interface Between a High-Performance Liquid Chromatograph and an Atomic Absorption Spectrophotometer, Clin. Chem., 27 (1981) 1546-1550.

152.   E.H. Hansen, Flow Injection Analysis: New Analytical Methods Based on the Use of Potentiometric and Spectrophotometric Flow-through Detectors, Anal. Proc. Div. Chem. Soc., 18 (1981) 261-264.

153.   J. Ruzicka, Theory and Principles of Flow Injection Analysis, Anal. Proc. Div. Chem. Soc., 18 (1981) 267-269.

154. L. Gorton and L. Ogren, Flow Injection Analysis for Glucose and Urea with Enzyme Reactors and On-Line Dialysis, Anal. Chim. Acta, 130 (1981) 45-53.

155. A.G. Fogg and D. Bhanot, Further Voltammetric Studies of Synthetic Food Coloring Matters at Glassy Carbon and Carbon Paste Electrodes Using Static and Flowing Systems, Analyst, 106 (1981) 883-889.

156. A.O. Jacintho, E.A.G. Zagatto, B.F. Reis, L.C.R. Pessenda and F.J. Krug, Merging Zones in Flow Injection Analysis. Part 6. Determination of Calcium in Natural Waters, Soil and Plant Materials with Glyoxal-bis-(2-hydroxyanil), Anal. Chim. Acta, 130 (1981) 361-368.

157. R. Kuroda and T. Mochizuki, Continuous Spectrophotometric Determination of Copper, Nickel, and Zinc in Copper-Base Alloys by Flow Injection Analysis, Talanta, 28 (1981) 389-393.

158. A.O. Jacintho, E.A.G. Zagatto, H. Bergamin Filho, F.J. Krug, B.F. Reis, R.E. Bruns and B.R. Kowalski, Flow Injection Systems with Inductively-Coupled Argon Plasma Atomic Emission Spectrometry. Part 1. Fundamental Considerations, Anal. Chim. Acta, 130 (1981) 243-255.

159. F.J. Krug, L.C.R. Pessenda, E.A.G. Zagatto, A.O. Jacintho and B.F. Reis, Spectrophotometric Flow Injection Determination of Chloride in Ethanol, Anal. Chim. Acta, 130 (1981) 409-413.

160. H.B. Hanekamp, P. Bos and D. Vittori, The Applicability of Phase-Sensitive Alternating Current Measurements in Flow-through Detection, Anal. Chim. Acta, 131 (1981) 149-158.

161. J.G. Williams, M. Holmes and D.G. Porter, Titrations of Spoilt Beer Samples by Flow-Injection Analysis, J. Autom. Chem., 4 (1982) 176-182.

162. T.J. Sly, D. Betteridge and D. Wibberley, An Improved Flow-through Phototransducer, J. Autom. Chem., 4 (1982) 186-189.

163. S.M. Ramasamy and H.A. Mottola, Repetitive Determinations of Sulfur Dioxide Gas in Air Samples by Flow Injection and Chemical Reaction at a Gas-Liquid Interface, Anal. Chem., 54 (1982) 283-286.

164. S.F. Simpson and F.J. Holler, Potentiometric Detection System for Flow Injection Titrimetry, Anal. Chem., 54 (1982) 43-46.

165. D.C. Shelly, T.M. Rossi and I.M. Warner, Multiple Solvent Extraction System with Flow Injection Technology, Anal. Chem., 54 (1982) 87-91.

166. H.A. Mottola, Continuous Flow Analysis Revisited, Anal. Chem., 53 (1981) 1312A-1316A.

167. A. Ivaska and T. Ryan, Application of a Voltammetric Flow-through Cell to Flow-Injection-Analysis (FIA), Coll. Czech. Chem. Commun., 46 (1981) 2865-2870.

168. K.K. Stewart, G.R. Beecher and P.E. Hare, Rapid Analysis of Discrete Samples: The Use of Nonsegmented, Continuous Flow, Anal. Biochem., 70 (1976) 167-173.

169. N. Yoza, Y. Hirai and S. Ohashi, Optimization of High-Performance Liquid Chromatography of Inorganic Polyphosphates for Routine Analysis, J. Chrom., 196 (1980) 471-480.

170. V.V.S. Eswara Dutt, A. Eskander-Hanna and H.A. Mottola, Kinetically Assisted Equilibrium-Based Repetitive Determination of Iron(II) with Ferrozine in Flow-through Systems, Anal. Chem., 48 (1976) 1207-1211.

171. J.F. Brown, K.K. Stewart and D.J. Higgs, Microcomputer Control and Data Systems for Automated Multiple Flow Injection Analysis, J. Autom. Chem., 3 (1981) 182-186.

172. L-G. Ekstrom, An Automated Method for Determination of Free Fatty Acids, J. Assoc. Off. Anal. Chem., 58 (1981) 935-938.

173. R. Playle, J. Gleed, R. Jonasson and J.R. Kramer, Comparison of Atomic Absorption Spectrometric, Spectrophotometric and Fluorimetric Methods for Determination of Aluminium in Water, Anal. Chim. Acta, 134 (1982) 369-373.

174. R. Eggli and R. Asper, Electrochemical Flow-through Detector for the Determination of Cysteine and Related Compounds, Anal. Chim. Acta, 101 (1978) 523-529.

175. O. Astrom, Flow Injection Analysis for the Determination of Bismuth by Atomic Absorption Spectrometry with Hydride Generation, Anal. Chem., 54 (1982) 190-193.

176. D. Betteridge and B. Fields, The Application of pH Gradients in Flow-Injection Analysis. A Method for Simultaneous Determination of Binary Mixtures of Metal Ions in Solution, Anal. Chim. Acta, 132 (1981) 139-152.

177. S. Hughes and D.C. Johnson, Amperometric Detection of Simple Carbohydrates at Platinum Electrodes in Alkaline Solutions by Application of a Triple-Pulse Potential Waveform, Anal. Chim. Acta, 132 (1981) 11-22.

178. J.F. Tyson and A.B. Idris, Flow Injection Sample Introduction for Atomic-Absorption Spectrometry: Applications of a Simplified Model for Dispersion, Analyst, 106 (1981) 1125-1129.

179. S. Hughes, P.L. Meschi and D.C. Johnson, Amperometric Detection of Simple Alcohols in Aqueous Solutions by Application of a Triple-Pulse Potential Waveform at Platinum Electrodes, Anal. Chim. Acta, 132 (1981) 1-10.

180. J.F. Van Staden, Automated Turbidimetric Determination of Sulfate in Surface, Ground, and Domestic Water by Flow-Injection Analysis, Fresenius' Z. Anal. Chem., 310 (1982) 239-242.

181. I. Kagevall, O. Astrom and A. Cedergren, Minimization of Interference Effects from Iodine-Consuming Samples in the Determination of Water with the Karl Fischer Reagent in a Flow Injection System, Anal. Chim. Acta, 132 (1981) 215-218.

182. K.K. Stewart, Flow-Injection Analysis: A Review of Its Early History, Talanta, 28 (1981) 789-797.

183. A.G. Fogg and N.K. Bsebsu, Differential-Pulse Voltammetric Determination of Phosphates as Molybdovanadophosphate at a Glassy Carbon Electrode and Assessment of Eluents for the Flow-Injection Voltammetry, Analyst, 106 (1981) 1288-1295.

184. T. Mochizuki and R. Kuroda, Determination of Beryllium in Copper-Beryllium Alloys by Flow-Injection Spectrophotometry, Fresenius' Z. Anal. Chem., 309 (1981) 363-364.

185. P.J. Worsfold, J. Ruzicka and E.H. Hansen, Rapid Automated Enzymatic Method for the Determination of Alcohol in Blood and Beverages Using Flow Injection Analysis, Analyst, 106 (1981) 1309-1317.

186. D.J. Leggett, N.H. Chen and D.S. Mahadevappa, Flow Injection Methods for Determination of Iron(III), Indian J. Chem., 20A (1981) 1051-1053.

187. B.S. Hui and C.O. Huber, Amperometric Detection of Amines and Amino Acids in Flow Injection Systems with a Nickel Oxide Electrode, Anal. Chim. Acta, 134 (1982) 211-218.

188. B.F. Rocks, R.A. Sherwood and C. Riley, Direct Determination of Therapeutic Concentrations of Lithium in Serum by Flow-Injection Analysis with Atomic Absorption Spectroscopic Detection, Clin. Chem., 28 (1982) 440-443.

189. J. Ruzicka, E.H. Hansen and A. Ramsing, Flow Injection Analyzer for Students, Teaching, and Research. Spectrophotometric Methods, Anal. Chim. Acta, 134 (1982) 55-71.

190. J.F. Tyson, Low Cost Continuous Flow Analysis. Flow-Injection Techniques in Atomic-Absorption Spectrometry, Anal. Proc. Div. Chem. Soc., 18 (1981) 542-545.

191. B.F. Rocks and C. Riley, Flow-Injection Analysis: A New Approach to Quantitative Measurements in Clinical Chemistry, Clin. Chem., 28 (1982) 409-421.

192. W.D. Basson, Consecutive Determination of Nitrogen, Phosphorous, and Calcium in Animal Feeds on a Single Channel Flow Injection Analyzer with a Common Analytical Manifold, Fresenius' Z. Anal. Chem., 311 (1982) 23-26.

193. H.F.R. Reinders, J.F. Van Staden and B. Griepink, Flow-through Determination of Sulphate in Water Using Different Methods: A Comparison, Fresenius' Z. Anal. Chem., 300 (1980) 273-276.

194. O. Kondo, H. Miyata and K. Toei, Determination of Sulfate in River Water by Flow Injection Analysis, Anal. Chim. Acta, 134 (1982) 353-358.

195. G. Svensson and T. Anfalt, Rapid Determination of Ammonia in Whole Blood and Plasma Using Flow Injection Analysis, Clin. Chim. Acta, 119 (1982) 7-14.

196. S. Olsen, J. Ruzicka and E.H. Hansen, Gradient Techniques in Flow Injection Analysis. Stopped-Flow Measurement of the Activity of Lactate Dehydrogenase with Electronic Dilution, Anal. Chim. Acta, 136 (1982) 101-112.

197. K.S. Johnson and R.L. Petty, Determination of Phosphate in Seawater by Flow Injection Analysis with Injection of Reagent, Anal. Chem., 54 (1982) 1185-1187.

198. M. Trojanowicz, W. Matuszewski and A. Hulanicki, Flow-Injection Potentiometric Determination of Residual Chlorine in Water, Anal. Chim. Acta, 136 (1982) 85-92.

199. T. Mochizuki and R. Kuroda, Rapid Continuous Determination of Aluminium in Copper-Base Alloys by Flow Injection Spectrophotometry, Fresenius' Z. Anal. Chem., 311 (1982) 11-15.

286

200.   W.E. Van der Linden, Flow Injection Analysis: The Manipulation of Dispersion, Trends in Anal. Chem., 1 (1982) 188-191.

201.   T.A. Kelly and G.D. Christian, Capillary Flow Injection Analysis for Enzyme Assay with Fluorescence Detection, Anal. Chem., 54 (1982) 1444-1445.

202.   U. Rydevik, L. Nord and F. Ingman, Automated Lactate Determination by Flow Injection Analysis, Int. J. Sports Med., 3 (1982) 47-49.

203.   E.L. Gulberg, A.S. Attiyat and G.D. Christian, Amperometric Systems for the Determination of Oxidase Enzyme Dependent Reactions by Continuous Flow and Flow Injection Analysis, J. Autom. Chem., 2 (1980) 189-194.

204.   J. Ruzicka and E.H. Hansen, Flow-Injection Analysis and Its Early History, Talanta, 29 (1982) 157.

205.   B. Olsson, Determination of Hydrogen Peroxide in a Flow System with Microperoxidase as Catalyst for the Luminol Chemiluminescence Reaction, Anal. Chim. Acta, 136 (1982) 113-119.

206.   D.J. Leggett, N.H. Chen and D.S. Mahadevappa, Flow Injection Analysis of Aromatic Sulfonyl Haloamines, Fresenius' Z. Anal. Chem., 31 (1982) 687-690.

207.   C.B. Ranger, Rapid Sample Pretreatment and Analysis Using Automated FIA, Am. Lab., 14 (1982) 56-68.

208.   L. Fossey and F.F. Cantwell, Characterization of Solvent Extraction/Flow Injection Analysis with Constant Pressure Pumping and Determination of Procyclidine Hydrochloride in Tablets, Anal. Chem., 54 (1982) 1693-1697.

209.   U. Forsman and A. Karlsson, Polarographic Determination of Penicilloic Acid in Penicillin Preparations with a Flow-Injection System, Anal. Chim. Acta, 139 (1982) 133-142.

210.   J. Matysik, E. Soczewinski, E. Zminkowska-Halliop and M. Przegalinski, Determination of o-Diphenols by Flow Injection Analysis with an Amperometric Detector, Chem. Anal. (Warsaw), 26 (1981) 463-468.

211.   T. Yao, Y. Kobayashi and S. Musha, Flow Injection Analysis for L-lactate with Immobilized Lactate Dehydrogenase, Anal. Chim. Acta, 138 (1982) 81-85.

212.   J.B. Kafil and C.O. Huber, Flow Injection Sample Processing with Nickel Oxide Electrode Amperometric Detection of Amino Acids Separated by Ion-Exchange Chromatography, Anal. Chim. Acta, 139 (1982) 347-352.

213.   M. Trojanowicz and W. Matuszewski, Limitation of Linear Response in Flow-Injection Systems with Ion-Selective Electrodes, Anal. Chim. Acta, 138 (1982) 71-79.

214.   A.G. Fogg and N.K. Bsebsu, Flow Injection Voltammetric Determination of Phosphate: Direct Injection of Phosphate into Molybdate Reagent, Analyst, 107 (1982) 566-570.

215.   J.F. Van Staden, Automated Simultaneous Determination of Nitrate and Nitrite by Pre-Valve Reduction of Nitrate in a Flow-Injection System, Anal. Chim. Acta, 138 (1982) 403-408.

216.   K. Ogata, K. Taguchi and T. Inanari, Phase Separator for Flow Injection Analysis, Anal. Chem., 54 (1982) 2127-2129.

217.   J. Wang and H.D. Dewald, A Porous-Jet Flow-through Electrode, Talanta, 29 (1982) 453-456.

218.   W.S. Gardner and H.A. Vanderploeg, Microsample-Filtering Device for Liquid Chromatography or Flow Injection Analysis, Anal. Chem., 54 (1982) 2129-2134.

219.   T.M. Rossi, D.C. Shelly and I.M. Warner, Optimization of a Flow Injection Analysis System for Multiple Solvent Extraction, Anal. Chem., 54 (1982) 2056-2061.

220.   D. Betteridge, Flow Injection Analysis in The Teaching Laboratory, Fresenius' Z. Anal. Chem., 312 (1982) 441-443.

221.   J. Janata and J. Ruzicka, Combination of Flow Injection Analysis and Voltammetry, Anal. Chim. Acta, 139 (1982) 105-115.

222.   D.J. Leggett, N.H. Chen and D.S. Mahadevappa, Rapid Determination of Residual Chlorine by Flow Injection Analysis, Analyst, 107 (1982) 433-441.

223.   J. Mortatti, F.J. Krug, L.C.R. Pessenda, E.A.G. Zagatto and S.S. Jorgensen, Determination of Iron in Natural Waters and Plant Material with 1,10-Phenanthroline by Flow Injection Analysis, Analyst, 107 (1982) 659-663.

224.   J.F. Van Staden, Automated Prevalve Sample Filtration in Flow Injection Analysis: Determination of Sulfate in Water Removing Suspended Solids and Color Before Sampling, Fresenius' Z. Anal. Chem., 312 (1982) 438-440.

225.   T. Korenaga and H. Ikatsu, The Determination of Chemical Oxygen Demand in Wastewaters with Dichromate by Flow-Injection Analysis, Anal. Chim. Acta, 141 (1982) 301-309.

226.   J.M. Harris, Flow Injection of Ultratrace Level Samples into Laser-Based Detectors, Anal. Chem., 54 (1982) 2337-2340.

227.   K.K. Stewart and A.G. Rosenfeld, Exponential Dilution Chambers for Scale Expansion in Flow Injection Analysis, Anal. Chem., 54 (1982) 2368-2372.

228.   S. Nakahara, M. Yamada and S. Suzuki, Chemiluminescence for the Determination of Traces of Cobalt(II) by Continuous Flow and Flow Injections Methods, Anal. Chim. Acta, 141 (1982) 255-262.

229.   J. Ruzicka and A. Ramsing, Flow Injection Analysis Using Ion-Sensitive Field Effect Transistors. A Model System for Discrete Assays and Continuous in Vitro Monitoring of pH and pCa, Scand. J. Clin. Lab. Invest., Suppl., 42 (1982) 35-41.

230.   C. Wyganowski, S. Motomizu and K. Toei, Spectrophotometric Determination of Aluminium in River Water with Bromopyrogallol Red and n-Tetradecyltrimethylammonium Bromide by Flow Injection Analysis, Anal. Chim. Acta, 140 (1982) 313-317.

231.   W.L. Caudill, J.O. Howell and R.M. Wightman, Flow Rate Independent Amperometric Cell, Anal. Chem., 54 (1982) 2532-2535.

232.   T. Mochizuki, Y. Toda and R. Kuroda, Flow-Injection Analysis of Silicate Rocks for Total Iron and Aluminium, Talanta, 29 (1982) 659-662.

288

233. B.F. Rocks, R.A. Sherwood, L.M. Bayford and C. Riley, Zinc and Copper Determination in Microsamples of Serum by Flow Injection and Atomic Absorption Spectroscopy, Ann. Clin. Biochem., 19 (1982) 338-344.

234. G. Gerhardt and R.N. Adams, Determination of Diffusion Coefficients by Flow Injection Analysis, Anal. Chem., 54 (1982) 2618-2620.

235. C.H.P. Bruins, D.A. Doornbos and K. Brunt, The Hydrodynamics of the Amperometric Detector Flow Cell with a Rotating Disk Electrode, Anal. Chim. Acta, 140 (1982) 39-49.

236. B. Karlberg, Flow Injection Analysis, in Frei (Ed.), Chemical Derivatization Analysis, Vol. 2, Plenum, 1-42, 1982.

237. A.G. Fogg, N.K. Bsebsu and M.A. Abdalla, Flow Injection Voltammetric Determination of Nitrite by Reduction at a Glassy Carbon Electrode in an Acidic Bromide or Chloride Media, Analyst, 107 (1982) 1040-1046.

238. T. Mochizuki and R. Kuroda, Flow Injection Analysis of Silicate Rocks Titanium, Analyst, 107 (1982) 1255-1260.

239. K. Brunt, Comparison Between the Performances of an Electrochemical Detector Flow Cell in a Potentiometric and an Amperometric Measuring System Using Glucose as a Test Compound, Analyst, 107 (1982) 1261-1271.

240. L. Nord and B. Karlberg, An Automated Extraction System for Flame Atomic Absorption Spectrometry, Anal. Chim. Acta, 125 (1981) 199-202.

241. C. Ridder, E.H. Hansen and J. Ruzicka, Flow Injection Analysis of Glucose in Human Serum by Chemiluminescence, Anal. Lett., 15 (1982) 1751-1766.

242. T. Ito, E. Nakagawa, H. Kawaguchi and A. Mizuike, Semi-Automatic Microliter Sample Injection into an Inductively Coupled Plasma for Simultaneous Multielement Analysis, Mikrochim. Acta, 1 (1982) 423-431.

243. C.M. Wolff and H.A. Mottola, Nonmebrane Amperometric Sensor for Dissolved Oxygen in Flow-through Systems, Anal. Chem., 49 (1977) 2118-2121.

244. S.M. Ramasamy, A. Iob and H.A. Mottola, Catalytic Determination of Enzymes and Metal Ions by Sample Injection in Closed-Loop Flow Systems, Anal. Chem., 51 (1979) 1637-1639.

245. V.V.S. Eswara Dutt and H.A. Mottola, Repitition Determination of Isonicotinic Acid Hydrazide in Flow-through Systems by Series Reactions, Anal. Chem., 49 (1977) 776-779.

246. V.V.S. Eswara Dutt, D. Scheeler and H.A. Mottola, Repetitive Determination of Iron(III) in Closed Flow-through Systems by Series Reactions, Anal. Chim. Acta, 94 (1977) 289-296.

247. V.V.S. Eswara Dutt and H.A. Mottola, Novel Approach to Reaction-Rate Based Determinations by Use of Transient Redox Effects, Anal. Chem., 47 (1975) 357-359.

248. V.V.S. Eswara Dutt and H.A. Mottola, Kinetics of Oxalate Attack on Ferrozine-Iron(II) Complex and Regeneration of Ligand in Determination of Iron(II), Anal. Chem., 49 (1977) 319-321.

249.   C.M. Wolff and H.A. Mottola, Enzymatic Substrate Determination in Closed Flow-through Systems by Sample Injection and Amperometric Monitoring of Dissolved Oxygen Levels, Anal. Chem., 50 (1978) 94-98.

250.   D.P. Nikolelis and H.A. Mottola, Repetitive Determinations of Amylase Maltose, Sucrose, and Lactose by Sample Injection Closed Flow-through Systems, Anal. Chem., 50 (1978) 1665-1670.

251.   A. Iob and H.A. Mottola, Continuous Flow Analysis for Uric Acid in Biological Fluids, with Immobilized Uricase in a Closed-Loop System, Anal. Chem., 52 (1980) 2332-2336.

252.   M. Yamada and S. Suzuki, Chemiluminescent Determination of Traces of Copper(II) by Flow Injection Method, Chem. Lett., 11 (1982) 1747-1748.

253.   R.L. Petty, W.C. Michel, J.P. Snow and K.S. Johnson, Determination of Total Primary Amines in Seawater and Plant Nectar with Flow Injection Sample Processing and Fluorescence Detection, Anal. Chim. Acta, 142 (1982) 299-304.

254.   S.A. McClintock and W.C. Purdy, A Microprocessor Controlled Potentiometric Detection System, Anal. Lett., 15 (1982) 1001-1008.

255.   A. Hu, R.E. Dessy and A. Graneli, Potentiometric Stripping with Matrix Exchange Techniques in Flow Injection Analysis of Heavy Metals in Groundwaters, Anal. Chem., 55 (1983) 320-328.

256.   A.G. Fogg, N.K. Bsebsu and M.A. Abdalla, Indirect Flow-Injection Voltammetric Determination of Aromatic Amines by Monitoring at a Glassy Carbon Electrode the Excess of Nitrite Remaining after Their Diazotization, Analyst, 107 (1982) 1462-1465.

257.   D.J. Hooley and R.E. Dessy, Continuous Flow Kinetic Techniques in Flow Injection Analysis, Anal. Chem., 55 (1983) 313-320.

258.   M. Thompson and U.J. Krull, Bilayer Lipid Membrane Electrochemistry in a Flow Injection System, Anal. Chim. Acta, 142 (1982) 207-216.

259.   A. Haemmerli, J. Janata and H.M. Brown, A Flow Injection System for Measurement of Chemical Response Time of Microelectrodes, Anal. Chim. Acta, 144 (1982) 115-121.

260.   Z. Fang and S. Xu, Determination of Molybdenum at μg/l Levels by Catalytic Spectrophotometric Flow Injection Analysis, Anal. Chim. Acta, 145 (1983) 143-150.

261.   G. Johansson, L. Ogren and B. Olsson, Enzyme Reactors in Unsegmented Flow Injection Analysis, Anal. Chim. Acta, 145 (1983) 71-85.

262.   J. Wang and H.D. Dewald, Flow-Injection Analysis of Oxidizable Species with Reverse-Pulse Amperometric Detection, Talanta, 29 (1982) 901-904.

263.   F.J. Krug, E.A.G. Zagatto, B.F. Reis, F.O. Bahia, A.O. Jacintho and S.S. Jorgensen, Turbidimetric Determination of Sulphate in Plant Digests and Natural Waters by Flow Injection Analysis with Alternating Streams, Anal. Chim. Acta, 145 (1983) 179-187.

264.   B. Olsson, L. Ogren and G. Johansson, An Enzyme Flow Injection Method for the Determination of Oxygen, Anal. Chim. Acta, 145 (1983) 101-108.

265. C. Riley, L.H. Aslett, B.F. Rocks, R.A. Sherwood, J.D.M. Watson and J. Morgon, Controlled Dispersion Analysis: Flow-Injection Analysis without Injection, Clin. Chem., 29 (1983) 332-335.

266. P. Van den Winkel, G. De Backer, M. Vandeputte, N. Mertens, L. Dryon and D.L. Massart, Performance and Characteristics of the Fluoride-Selective Electrode in a Flow Injection System, Anal. Chim. Acta, 145 (1983) 207-212.

267. P. J. Worsfold, The Bio-Analytical Potential of Flow Injection Analysis, Anal. Chim. Acta, 145 (1983) 117-124.

268. M. Strandberg and S. Thelander, A Microprocessor-Controlled Flow Injection Analyzer for the Determination of Terbutaline Sulphate, Anal. Chim. Acta, 145 (1983) 219-223.

269. T.A. Kelly and G.D. Christian, Homogeneous Enzymatic Fluorescence Immunoassay of Serum IgG by Continuous Flow-Injection Analysis, Talanta, 29 (1982) 1109-1112.

270. V.K. Mahant, J.N. Miller and H. Thakrar, Flow Injection Analysis with Chemiluminescence Detection in the Determination of Fluorescein- and Fluorescamine-Labeled Species, Anal. Chim. Acta, 145 (1983) 203-206.

271. C.B. Ranger, Flow-Injection Analysis: A New Approach to Near-Real-Time Process Monitoring, Autom. Stream Anal. Process Control, 1 (1982) 39-66.

272. J. Ruzicka and E.H. Hansen, Recent Developments in Flow Injection Analysis: Gradient Techniques and Hydrodynamic Injection, Anal. Chim. Acta, 145 (1983) 1-15.

273. J.M. Reijn, H. Poppe and W.E. Van der Linden, A Possible Approach to the Optimization of Flow Injection Analysis, Anal. Chim. Acta, 145 (1983) 59-70.

274. J.F. Tyson, J.M.H. Appleton and A.B. Idris, Flow Injection Calibration Methods for Atomic Absorption Spectrometry, Anal. Chim. Acta, 145 (1983) 159-168.

275. T.J. Sly, D. Betteridge, D. Wibberley and D.G. Porter, An Improved Flow-through Transducer, J. Autom. Chem., 4 (1982) 186-189.

276. H. Poppe, The Performance of Some Liquid Phase Flow-through Detectors, Anal. Chim. Acta, 145 (1983) 17-26.

277. E.A.G. Zagatto, A.O. Jacintho, F.J. Krug, B.F. Reis, R.E. Bruns and M.C.U. Araujo, Flow Injection Systems with Inductively-Coupled Argon Plasma Atomic Emission Spectrometry. Part 2. The Generalized, Standard Addition Method, Anal. Chim. Acta, 145 (1983) 169-178.

278. J. Wang, H.D. Dewald and B. Greene, Anodic Stripping Voltammetry of Heavy Metals with a Flow Injection System, Anal. Chim. Acta, 146 (1983) 45-50.

279. H. Kagenow and A. Jensen, Kinetic Determination of Magnesium and Calcium by Stopped-Flow Injection Analysis, Anal. Chim. Acta, 145 (1983) 125-133.

280. G. Nakagawa, H. Wada and C. Wei, Spectrophotometric Determination of Calcium with a Flow Injection System, Anal. Chim. Acta, 145 (1983) 135-141.

281.   H. Lundback, Amperometric Determination of Hydrogen Peroxide in Pickling Baths for Copper and Copper Alloys by Flow Injection Analysis, Anal. Chim. Acta, 145 (1983) 189-196.

282.   B. Olsson and L. Ogren, Optimization of Peroxide Immobilization and of the Design of Packed-Bed Enzyme Reactors for Flow Injection Analysis, Anal. Chim. Acta, 145 (1983) 87-99.

283.   D. Betteridge, W.C. Cheng, E.L. Dagless, P. David, T.B. Goad, D.R. Deans, D.A. Newton and T.B. Pierce, An Automated Viscometer Based on High-Precision Flow Injection Analysis. Part I. Apparatus for High-Precision Flow Injection Analysis, Analyst, 108 (1983) 1-16.

284.   D. Betteridge, W.C. Cheng, E.L. Dagless, P. David, T.B. Goad, D.R. Deans, D.A. Newton and T.B. Pierce, An Automated Viscometer Based on High-Precision Flow Injection Analysis. Part II. Measurement of Viscosity and Diffusion Coefficients, Analyst, 108 (1983) 17-32.

285.   R.C. Schothorst, J.M. Reijn, H. Poppe, and G. Den Boef, The Application of Strongly Reducing Agents in Flow Injection Analysis. Part I. Chromium(II) and Vanadium(II), Anal. Chim. Acta, 145 (1983) 197-201.

286.   L. Nord and B. Karlberg, Sample Preconcentration by Continuous Flow Extraction with a Flow Injection Atomic Absorption Detection System, Anal. Chim. Acta, 145 (1983) 151-158.

287.   K. Honda, J. Sekino and K. Imai, Bis(2,4-dinitrophenyl) Oxalate as a Chemiluminescence Reagent. Determination of Fluorescent Compounds by Flow Injection Analysis, Anal. Chem., 55 (1983) 940-943.

288.   T. Yamane and H.A. Mottola, The Transient Oxidation of Brucine in Solution as a Tool for the Determination of Chromium(VI) and Brucine, Anal. Chim. Acta, 146 (1983) 181-190.

289.   A.G. Fogg, A.Y. Chasmi and M.A. Abdalla, Flow Injection Voltammetric Determination of Nitrate after Reduction to Nitrite, Analyst, 108 (1983) 464-469.

290.   T.P. Lynch, A.F. Taylor and J.N. Wilson, Fully Automatic Flow Injection System for the Determination of Uranium at Trace Levels in Ore Leachates, Analyst, 108 (1983) 470-475.

291.   M. Yamada, T. Nakada and S. Suzuki, The Determination of Sulfite in a Flow Injection System with Chemiluminescence Detection, Anal. Chim. Acta, 147 (1983) 401-404.

292.   K.W. Pratt and D.C. Johnson, The Vibrating Wire Electrode as an Amperometric Detector for Flow-Injection Systems, Anal. Chim. Acta, 148 (1983) 87-101.

293.   P.W. Alexander and U. Akapongkul, Amperometric Determination of Metal Ions in a Flow Injection System with a Copper-Amalgam Electrode, Anal. Chim. Acta, 148 (1983) 103-109.

294.   E.H. Hansen, J. Ruzicka, F.J. Krug and E.A.G. Zagatto, Selectivity in Flow Injection Analysis, Anal. Chim. Acta, 148 (1983) 111-125.

292

295. G.L. Abdullahi, J.N. Miller, H.N. Sturley and J.W. Bridges, Studies of Drug-Protein Binding Interactions by Flow Injection Analysis with Fluorometric Detection, Anal. Chim. Acta, 145 (1983) 109-116.

296. H.A. Mottola, Enzymatic Preparations in Analytical Continuous-Flow Systems, Anal. Chim. Acta, 145 (1983) 27-39.

297. P.W. Alexander, R.J. Finlayson, L.E. Smythe and A. Thalib, Rapid Flow Analysis with Inductively-Coupled Plasma Atomic-Emission Spectroscopy Using a Micro-Injection Technique, Analyst, 107 (1982) 1335-1342.

298. D. Pilosof and T.A. Nieman, Microporous Membrane Flow Cell with Nonimmobilized Enzyme for Chemiluminescent Determination of Glucose, Anal. Chem., 54 (1982) 1698-1701.

299. C. Riley, B.F. Rocks, R.A. Sherwood, L.H. Aslett and P.R. Oldfield, A Stopped-Flow/Flow Injection System for Automation of Alpha 2 Macroglobulin Kinetic Studies, J. Autom. Chem., 5 (1983) 32-35.

300. J. Karlsson, I. Jacobs, B. Sjodin, P. Tesch, P. Kaiser, O. Sahl and B. Karlberg, Semi-Automatic Blood Lactate Assay: Experiments from an Exercise Laboratory, Int. J. Sports Med., 4 (1983) 52-55.

301. P.W. Alexander and A. Thalib, Nonsegmented Rapid-Flow Analysis with Ultraviolet/Visible Spectrophotometric Determination for Short Sampling Times, Anal. Chem., 55 (1983) 497-501.

302. J.L. Burguera, M. Burguera, M. Gallignani and O.M. Alarcon, More on Flow Injection/Atomic Absorption Analysis for Electrolytes, Clin. Chem., 29 (1983) 568-569.

303. B.F. Rocks, R.A. Sherwood and C. Riley, More on Flow Injection/Atomic Absorption Analysis for Electrolytes. Comments, Clin. Chem., 29 (1983) 569-570.

304. B.F. Rocks, R.A. Sherwood, Z.J. Turner and C. Riley, Serum Iron and Total Iron-Binding Capacity Determination by Flow-Injection Analysis with Atomic Absorption Detection, Ann. Clin. Biochem., 20 (1983) 72-76.

305. D. Betteridge and B. Fields, Two-Point Kinetic Simultaneous Determination of Cobalt(II) and Nickel(II) in Aqueous Solution Using Flow Injection Analysis (FIA), Fresenius' Z. Anal. Chem., 314 (1983) 386-390.

306. J.F. Tyson, J.M.H. Appleton and A.B. Idris, Flow Injection Sample Introduction Methods for Atomic Absorption Spectrometry, Analyst, 108 (1983) 153-158.

307. B. Karlberg, Automation of Wet Chemical Procedures Using FIA, Am. Lab., 15 (1983) 73-76.

308. P. Marstorp, T. Anfalt and L. Andersson, Determination of Oxidized Ketone Bodies in Milk by Flow Injection Analysis, Anal. Chim. Acta, 149 (1983) 281-289.

309. J.F. Van Staden, Simultaneous Determination of Protein (Nitrogen), Phosphorus, and Calcium in Animal Feeds by Multichannel Flow Injection Analysis, J. Assoc. Off. Anal. Chem., 66 (1983) 718-726.

310. D. Betteridge, T.J. Sly, A.P. Wade and J.E.W. Tillman, Computer-Assisted Optimization for Flow Injection Analysis of Isoprenaline, Anal. Chem., 55 (1983) 1292-1299.

311. S. Greenfield, Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES) with Flow Injection Analysis (FIA), Spectrochim. Acta, 38B (1983) 93-105.

312. J. Wang and B.A. Freiha, Selective Voltammetric Detection Based on Adsorptive Preconcentration for Flow Injection Analysis, Anal. Chem., 55 (1983) 1285-1288.

313. U.J. Krull and M. Thompson, Flow Injection Analysis with a Bilayer Lipid Membrane Detector, Trends in Anal. Chem., 2 (1983), VI-VII.

314. H. Wada, A. Yuchi and G. Nakagawa, Spectrophotometric Determination of Magnesium by Flow Injection Analysis with a Ligand Buffer for Masking Calcium, Anal. Chim. Acta, 149 (1983) 291-296.

315. T. Hara, M. Toriyama and K. Tsukagoshi, Determination of a Small Amount of Biological Constituent by Use of Chemiluminescence. I. The Flow Injection Analysis of Protein, Bull. Chem. Soc. Jpn., 56 (1983) 1382-1387.

316. L.C. Davis and G.A. Radke, Chemically Coupled Spectrophotometric Assays Based on Flow Injection Analysis: Determination of Nitrogenase by Assays for Creatine, Ammonia, Hydrazine, Phosphate, and Dithionite, Anal. Biochem., 140 (1984) 434-442.

317. K. Ogata, S. Tanabe and T. Imanari, Flame Atomic Absorption Spectrophotometry Coupled with Solvent Extraction/Flow Injection Analysis, Chem. Pharm. Bull., 31 (1983) 1419-1421.

318. D.J. Leggett, N.H. Chen and D.S. Mahadevappa, A Flow Injection Method for Analysis of Residual Chlorine by the DPD Procedure, Fresenius' Z. Anal. Chem., 315 (1983) 47-50.

319. J.F. Van Staden, Determination of Creatinine in Urine and Serum by Flow Injection Analysis Using the Jaffe Reaction, Fresenius' Z. Anal. Chem., 315 (1983) 141-144.

320. F.J. Krug, B.F. Reis, M.F. Gine, E.A.G. Zagatto, J.R. Ferreira and A.O. Jacintho, Zone Trapping in Flow Injection Analysis. Spectrophotometric Determination of Low Levels of Ammonium Ion in Natural Waters, Anal. Chim. Acta, 151 (1983) 39-48.

321. M. Trojanowicz and W. Matuszewski, Potentiometric Flow Injection Determination of Chloride, Anal. Chim. Acta, 151 (1983) 77-85.

322. K.K. Stewart, Flow Injection Analysis. New Tool for Old Assays. New Approach to Analytical Measurements, Anal. Chem., 55 (1983) 931A-940A.

323. R.A. Leach, J. Ruzicka and J.M. Harris, Spectrophotometric Determination of Metals at Trace Levels by Flow Injection and Series Differential Detection, Anal. Chem., 55 (1983) 1669-1673.

324. A.P. Wade, Computer Assisted Optimization of Chemical Systems, in Particular Flow Injection Analysis, Proc. Anal. Div. Chem. Soc., 20 (1983) 108-111.

294

325.   J. Wang and B.A. Freiha, Flow Electrolysis at a Porous Tubular Electrode with Internal Stirring, Anal. Chim. Acta, 151 (1983) 109-116.

326.   L. Fossey and F.F. Cantwell, Simultaneous Monitoring of Both Phases in the Solvent Extraction/Flow Injection Analysis of Dramamine Tablets, Anal. Chem., 55 (1983) 1882-1885.

327.   N. Thoegersen, J. Janata and J. Ruzicka, Flow Injection Analysis and Cyclic Voltammetry, Anal. Chem., 55 (1983) 1986-1988.

328.   J.J. Donkerbroek, A.C. Veltkamp, C. Gooijer, N.H. Velthorst and R.W. Frei, Quenched Room Temperature Phosphorescence Detection for Flow Injection and Liquid Chromatography, Anal. Chem., 55 (1983) 1886-1893.

329.   W.L. Caudill, A.G. Ewing, S. Jones and R.M. Wightman, Liquid Chromatography with Rapid Scanning Electrochemical Detection at Carbon Electrodes, Anal. Chem., 55 (1983) 1877-1881.

330.   J.L. Burguera, M. Burguera and M. Gallignani, Direct Determination of Sodium and Potassium in Blood Serum by Flow Injection and Atomic Absorption Spectrophotometry, An. Acad. Bras. Cienc., 55 (1983) 209-211.

331.   W.E. Van der Linden, Membrane Separation in Flow Injection Analysis. Gas-Diffusion, Anal. Chim. Acta, 151 (1983) 359-369.

332.   J. Ruzicka, Flow Injection Analysis. From Test Tube to Integrated Microconduits, Anal. Chem., 55 (1983) 1040A-1053A.

333.   K. Matsumoto, H. Ukeda and Y. Osajima, Flow Injection Analysis of Reduced Nicotinamide Adenine Dinucleotide Using Beta-Naphthoquinone-4-Sulfonate as a Mediator, Agric. Biol. Chem., 48 (1984) 1879-1880.

334.   K.L. Lu and Y.M. Chen, Catalytic Photometric Method for the Determination of Molybdenum in Plants with Flow Injection Analysis, Proc. Natl. Sci. Counc., 8 (1984) 85-92.

335.   S. Motomizu, T. Wakimoto and K. Toei, Determination of Trace Amounts of Phosphate in River Water by Flow Injection Analysis, Talanta, 30 (1983) 333-338.

336.   J.C. De Andrade, J.C. Rocha, C. Pasquini and N. Baccan, Effect of On-Line Complex Formation Kinetics on the Flow Injection Analysis Signal: The Spectrophotometric Determination of Chromium(VI), Analyst, 108 (1983) 621-625.

337.   M.E. Meyerhoff and P.M. Kovach, An Ion-Selective Electrode/Flow Injection Analysis Experiment: Determination of Potassium in Serum, J. Chem. Educ., 60 (1983) 766-768.

338.   M.H. Shah and J.T. Stewart, Amperometric Determination of Isoniazid in a Flowing Stream at the Glassy Carbon Electrode, Anal. Lett., 16 (1983) 913-923.

339.   G.B. Marshall and D. Midgley, Potentiometric Determination of Sulfite by Use of Mercury(I) Chloride - Mercury(II) Sulfide Electrodes in Flow Injection Analysis and in Air-Gap Electrodes, Analyst, 108 (1983) 701-711.

340. J.L. Burguera and M. Burguera, The Principle, Applications and Trends of Flow Injection Analysis for Monitoring Chemiluminescence Reactions, Acta. Cient. Venez., 34 (1983) 79-83.

341. A.G. Fogg, M.A. Ali and M.A. Abdalla, Online Bromometric Determination of Phenol, Analine, Aspirin, and Isoniazid Using Flow Injection Voltammetry, Analyst, 108 (1983) 840-846.

342. N. Yoza, H. Hirano, M. Okamura, S. Ohashi, Y. Hirai and K. Tomokuni, Measurement of Enzymic Activity of Inorganic Pyrophosphatase for Pyrophosphate by Flow Injection Analysis, Chem. Lett., 9 (1983) 1433-1436.

343. J. Thomsen, K.S. Johnson and R.L. Petty, Determination of Reactive Silicate in Seawater by Flow Injection Analysis, Anal. Chem., 55 (1983) 2378-2382.

344. J.J. Harrow and J. Janata, Comparison of Sample Injection Systems for Flow Injection Analysis, Anal. Chem., 55 (1983) 2461-2464.

345. J.A. Polta and D.C. Johnson, The Direct Electrochemical Detection of Amino Acids at a Platinum Electrode in an Alkaline Chromatographic Effluent, J. Liq. Chromatogr., 6 (1983) 1727-1743.

346. C.W. Bradberry and R.N. Adams, Flow Injection Analysis with an Enzyme Reactor Bed for Determination of Ascorbic Acid in Brain Tissue, Anal. Chem., 55 (1983) 2439-2440.

347. S. Olsen, L.C.R. Pessenda, J. Ruzicka and E.H. Hansen, Combination of Flow Injection Analysis with Flame Atomic - Absorption Spectrophotometry: Determination of Trace Amounts of Heavy Metals in Polluted Seawater, Analyst, 108 (1983) 905-917.

348. J.L. Burguera and M. Burguera, Determination of Cadmium in Human Urine by Extraction with Dithizone in a Flow Injection System, Anal. Chim. Acta, 153 (1983) 207-212.

349. P.J. Worsfold, Enzymic Assays Using Flow Injection Analysis, Proc. Anal. Div. Chem. Soc., 20 (1983), 486-491.

350. P. Roehrig, C.M. Wolff and J.P. Schwing, Repetitive Enzymatic Determination of Glucose with Regeneration and Recycling of Coenzyme and Enzymes, Anal. Chim. Acta, 153 (1983) 181-190.

351. M. Van Son, R.C. Schothorst and G. Den Boef, Determination of Total Ammoniacal Nitrogen in Water by Flow Injection Analysis and a Gas-Diffusion Membrane, Anal. Chim. Acta, 153 (1983) 271-275.

352. J.F. Tyson, Flow Injection Methods and Atomic-Absorption Spectrophotometry, Proc. Anal. Div. Chem. Soc., 20 (1983) 488-491.

353. R.C. Schothorst and G. Den Boef, The Application of Strongly Reducing Agents in Flow Injection Analysis. Part 2. Chromium(II), Anal. Chim. Acta, 153 (1983) 133-139.

354. T.E. Edmonds and G. Coutts, Flow Injection Analysis System for Determining Soil pH, Analyst, 108 (1983) 1013-1017.

355. H. Wada, T. Ishuzuki and G. Nakagawa, Synthesis of 2-(2-Thiazolylazo)-4-methyl-5-(sulfopropylamino)benzoic Acid and the Application to the Flow Injection Analysis of Copper(II), Mikrochim. Acta, 3 (1983) 235-244.

296

356.   N. Zhou, W. Frech and E. Lundberg, Rapid Determination of Lead, Bismuth, Antimony and Silver in Steels by Flame Atomic Absorption Spectrometry Combined with Flow Injection Analysis, Anal. Chim. Acta, 153 (1983) 23-31.

357.   T. Yao, Y. Kobayashi and M. Sato, Amperometric Determination of Phospholipids in Blood Serum with a Lecithin-Sensitive Electrode in a Flow Injection System, Anal. Chim. Acta, 153 (1983) 337-340.

358.   H. Wada, G. Nakagawa and K. Ohshita, Spectrophotometric Determination of Traces of Iron with 2-(3,5-Dibromo-2-pyridylazo)-5-(N-ethyl-N-(3-sulfopropyl)amino)phenol and Its Application in FIA, Anal. Chim. Acta, 153 (1983) 199-206.

359.   J. Wang and H.D. Dewald, Potential Scanning Voltammetric Detection for Flow Injection Systems, Anal. Chim. Acta, 153 (1983) 325-330.

360.   H.W. Van Rooijen and H. Poppe, Noise and Drift Phenomena in Amperometric and Coulometric Detectors for HPLC and FIA, J. Liq. Chromatogr., 6 (1983) 2231-2254.

361.   K. Kusube, K. Abe, O. Hiroshima and Y. Ishiguro, Electrochemical Derivatization of Thiamin in a Flow Injection System - Application to Thiamin Analysis, Chem. Pharm. Bull., 31 (1983) 3589-3594.

362.   H. Morita, T. Kimoto and S. Shimomura, Flow Injection Analysis of Mercury/Cold Vapor Atomic Fluorescence Spectrophotometry, Anal. Lett., 16 (1983) 1187-1195.

363.   A.P. Wade, Optimization of Flow Injection Analysis and Polarography by the Modified Simplex Method, Proc. Anal. Div. Chem. Soc., 20 (1983) 523-527.

364.   G. Decristoforo and B. Danielsson, Flow Injection Analysis with Enzyme Thermistor Detector for Automated Determination of Beta-Lactams, Anal. Chem., 56 (1984) 263-268.

365.   A.G. Fogg, G.C. Cripps and B.J. Birch, Static and Flow Injection Voltammetric Determination of Total Phosphate and Soluble Silicate in Commercial Washing Powders at a Glassy Carbon Electrode, Analyst, 108 (1983) 1485-1489.

366.   A.J. Frend, G.J. Moody, J.D.R. Thomas and B.J. Birch, Flow Injection Analysis with Tubular Membrane Ion-Selective Electrodes in the Presence of Anionic Surfactants, Analyst, 108 (1983) 1357-1364.

367.   J.T. Vanderslice, G.R. Beecher and A.G. Rosenfeld, Determination of First-Order Reaction Rate Constants by Flow Injection Analysis, Anal. Chem., 56 (1984) 268-270.

368.   K.E. Lawrence, G.W. Rice and V.A. Fassel, Direct Liquid Sample Introduction for Flow Injection Analysis and Liquid Chromatography with Inductively Coupled, Argon Plasma Spectrometric Detection, Anal. Chem., 56 (1984) 289-292.

369.   J. Wang and H.D. Dewald, Substractive Anodic Stripping Voltammetry with Flow Injection Analysis, Anal. Chem., 56 (1984) 156-159.

370.   G.C.M. Bourke, G. Stedman and A.P. Wade, The Spectrophotometric Determination of Hydroxylamine Alone and in the Presence of Hydrazine by Flow Injection Analysis, Anal. Chim. Acta, 153 (1983) 277-280.

371. O.F. Kamson and A. Townshend, Ion-Exchange Removal of Some Interferences on the Determination of Calcium by Flow Injection Analysis and Atomic Absorption Spectrometry, Anal. Chim. Acta, 155 (1983) 253-257.

372. M. Yamada, T. Komatsu, S. Nakahara and S. Suzuki, Improved Chemiluminescence Determination of Traces of Cobalt(II) by Continuous Flow and Flow Injection Methods, Anal. Chim. Acta, 155 (1983) 259-262.

373. J.T. Vanderslice, G.R. Beecher and A.G. Rosenfeld, Dispersion and Diffusion Coefficients in Flow Injection Analysis, Anal. Chem., 56 (1984) 292-293.

374. T. Yao, Flow Injection Analysis for Cholinesterase in Blood Serum by Use of a Choline-Sensitive Electrode as an Amperometric Detector, Anal. Chim. Acta, 153 (1983) 169-174.

375. S. Nakashima, M. Yagi, M. Zenki, A. Takahashi and K. Toei, Spectrophotometric Determination of Nitrite in Natural Waters by Flow Injection Analysis, Anal. Chim. Acta, 155 (1983) 263-268.

376. J. Wang and B.A. Freiha, Preconcentration and Differential Pulse Voltammetry of Butylated Hydroxyanisole at a Carbon Paste Electrode, Anal. Chim. Acta, 154 (1983) 87-94.

377. B.P. Bubnis, M.R. Straka and G.E. Pacey, Metal Speciation by Flow Injection Analysis, Talanta, 30 (1983) 841-844.

378. M.F. Gine, B.F. Reis, E.A.G. Zagatto, F.J. Krug and A.O. Jacintho, A Simple Procedure for Standard Additions in Flow Injection Analysis. Spectrophotometric Determination of Nitrate in Plant Extracts, Anal. Chim. Acta, 155 (1983) 131-138.

379. K.S. Johnson and R.L. Petty, Determination of Nitrate and Nitrite in Seawater by Flow Injection Analysis, Limnol. Oceanogr., 28 (1983) 1260-1266.

380. T.A.H.M. Janse, P.F.A. Van der Wiel and G. Kateman, Experimental Optimization Procedures in the Determination of Phosphate by Flow Injection Analysis, Anal. Chim. Acta, 155 (1983) 89-102.

381. A.S. Attiyat and G.D. Christian, Nonaqueous Solvents as Carrier or Sample Solvent in Flow Injection Analysis/Atomic Absorption Spectrometry, Anal. Chem., 56 (1984) 439-442.

382. D. MacKoul, D.C. Johnson and K.G. Schick, Effect of Variation in Flow Rate on Amperometric Detection in Flow Injection Analysis, Anal. Chem., 56 (1984) 436-439.

383. J.C. De Andrade, C. Pasquini, N. Baccan and J.C. Van Loon, Cold Vapor Atomic Absorption Determination of Mercury by Flow Injection Analysis Using a Teflon Membrane Phase Separator Coupled to the Absorption Cell, Spectrochim. Acta, 38B (1983) 1329-1338.

384. M. Thompson, U.J. Krull and L. Bendell-Young, Biosensors and Their Uses in Flow Injection Systems, Proc. Anal. Div. Chem. Soc., 20 (1983) 568-569.

385. H. Lundback, G. Johansson and O. Holst, Determination of Hydrogen Peroxide for Application in Aerobic Cell Systems Oxygenated Via Hydrogen Peroxide, Anal. Chim. Acta, 155 (1983) 47-56.

386.   W.E. Van der Linden, The Optimum Composition of pH-Sensitive Acceptor Solutions for Membrane Separation in Flow Injection Analysis, Anal. Chim. Acta, 155 (1983) 273-277.

387.   D.J. Curran and T.P. Tougas, Electrochemical Detector Based on a Reticulated Vitreous Carbon Working Electrode for Liquid Chromatography and Flow Injection Analysis, Anal. Chem., 56 (1984) 672-678.

388.   G. Schulze, M. Husch and W. Frenzel, Flow Injection Potentiometric Stripping Analysis and Potentiometric Stripping Coulometry. Microanalytical Methods for Heavy Metals, Mikrochim. Acta, 1 (1984) 191-200.

389.   B.F. Rocks, R.A. Sherwood and C. Riley, Direct Determination of Calcium and Magnesium in Serum Using Flow Injection Analysis and Atomic Absorption Spectroscopy, Ann. Clin. Biochem., 21 (1984) 51-56.

390.   S. Nakashima, M. Yagi, M. Zenki and M. Doi, Determination of Sulfate in Natural Water by Flow Injection Analysis, Fresenius' Z. Anal. Chem., 317 (1984) 29-31.

391.   N. Yoza, T. Miyaji, Y. Hirai and S. Ohashi, Determination of Complexing Abilities of Ligands for Metal Ions by Flow Injection Analysis and High-Performance Liquid Chromatography. I. Principle of the Substitution Reaction Method, J. Chromatogr., 283 (1984) 89-98.

392.   E.A. Jones, Spectrophotometric Determination of Sulfate in Sodium Hydroxide Solutions by Flow Injection Analysis, Anal. Chim. Acta, 156 (1984) 313-317.

393.   T. Hara, M. Toriyama and K. Tsukagoshi, Determination of a Small Amount of a Biological Constituent by the Use of Chemiluminescence. II. Determination of Albumin as a Model Protein by Means of the FIA Using a Co(III) Complex Compound as a Catalyst, Bull. Chem. Soc. Jpn., 57 (1984) 389-390.

394.   M. Bos, J.H.H.G. Van Willigen and W.E. Van der Linden, Flow Injection Analysis with Tensammetric Detection for the Determination of Detergents, Anal. Chim. Acta, 156 (1984) 71-76.

395.   J.M. Reijn, H. Poppe and W.E. Van der Linden, Kinetics in a Single Bead String Reactor for Flow Injection Analysis, Anal. Chem., 56 (1984) 943-948.

396.   C. Pasquini and W.A. De Oliveira, Comparison of Merging Zones, Injection of Reagent and Single-Line Manifolds for Enthalpimetric Flow Injection Analysis, Anal. Chim. Acta, 156 (1984) 307-312.

397.   A.S. Attiyat and G.D. Christian, Flow Injection Analysis-Atomic Absorption Determination of Serum Zinc, Clin. Chim. Acta, 137 (1984) 151-157.

398.   D. Betteridge, N.G. Courtney, T.J. Sly and D.G. Porter, Development of a Flow Injection Analyzer for the Post-Column Detection of Sugars Separated by High-Performance Liquid Chromatography, Analyst, 109 (1984) 91-93.

399.   A.G. Fogg and N.K. Bsebsu, Sequential Flow Injection Voltammetric Determination of Phosphate and Nitrite by Injection of Reagents into a Sample Stream, Analyst, 109 (1984) 19-21.

400. C.C. Painton and H.A. Mottola, Kinetics in Continuous Flow Sample Processing. Chemical Contributions to Dispersion in Flow Injection Techniques, Anal. Chim. Acta, 158 (1984) 67-84.

401. J.F. Tyson and A.B. Idris, Determination of Chromium in Steel by Flame Atomic-Absorption Spectrometry Using a Flow Injection Standard Additions Method, Analyst, 109 (1984) 23-26.

402. H. Mueller, Determination of Iodide, Cyanide, Molybdenum(VI) and Tungsten(VI) with the Flow-through Iodide-Sensitive Electrode Using the Flow Injection Method, Anal. Chem. Symp. Ser., 18 (1984) 353-364.

403. L. Nondek, Band Broadening in Solid-Phase Derivatization Reactions for Irreversible First-Order Reactions, Anal. Chem., 56 (1984) 1192-1194.

404. A. Fernandez, M.D. Luque de Castro and M. Valcarcel, Comparison of Flow Injection Analysis Configurations for Differential Kinetic Determination of Cobalt and Nickel, Anal. Chem., 56 (1984) 1146-1151.

405. Y. Israel and R.M. Barnes, Standard Addition Method in Flow Injection Analysis with Inductively Coupled Plasma Emission Spectrometry, Anal. Chem., 56 (1984) 1188-1192.

406. J.L. Burguera and M. Burguera, Determination of Sulfur Anions by Flow Injection with a Molecular Emission Cavity Detector, Anal. Chim. Acta, 157 (1984) 177-181.

407. H.J. Wieck, G.H. Heider and A.M. Yacynych, Chemically Modified Reticulated Vitreous Carbon Electrode with Immobilized Enzyme as a Detector in Flow Injection Determination of Glucose, Anal. Chim. Acta, 158 (1984) 137-141.

408. H. Weicker, H. Haegele, B. Kornes and A. Werner, Determination of Alanine, Lactate, Pyruvate, Beta-hydroxybutyrate, and Acetoacetate by Flow Injection Analysis (FIA), Int. J. Sports Med., 5 (1984) 47-54.

409. J. Wang and B.A. Freiha, Thin Layer Flow Cell with a Rotating Disk Electrode, J. Electroanal. Chem., 164 (1984) 79-87.

410. J.F. Tyson, Extended Calibration of Flame Atomic-Absorption Instruments by a Flow Injection Peak Width Method, Analyst, 109 (1984) 319-321.

411. R.S. Brazell, R.W. Holmberg and J.H. Moneyhun, Application of High-Performance Liquid Chromatography-Flow Injection Analysis for the Determination of Polyphosphoric Acids in Phosphorus Smokes, J. Chromatogr., 290 (1984) 163-172.

412. T.P. Lynch, Determination of Free Cyanide in Mineral Leachates, Analyst, 109 (1984) 421-423.

413. M.R. Straka, G.E. Pacey and G. Gordon, Residual Ozone Determination by Flow Injection Analysis, Anal. Chem., 56 (1984) 1973-1975.

414. P.J. Worsfold and A. Hughes, A Model Immunoassay Using Automated Flow Injection Analysis, Analyst, 109 (1984) 339-341.

415. F.L. Boza, M.D. Luque de Castro and M. Valcarcel, Catalytic-Fluorimetric Determination of Copper at the Nanograms Per Millilitre Level by Flow Injection Analysis, Analyst, 109 (1984) 333-337.

416. P.J. Worsfold, A.G. Cox and C.W. McLeod, Simultaneous Multi-Element Analysis of Blood Serum by Flow Injection-Inductively Coupled Plasma Atomic Emission Spectrometry, Analyst, 109 (1984) 327-332.

417. K. Backstrom, L.-G. Danielsson and L. Nord, Sample Work-up for Graphite Furnace Atomic-Absorption Spectrometry Using Continuous Flow Extraction, Analyst, 109 (1984) 323-325.

418. M.D. Luque de Castro and M. Valcarcel, Simultaneous Determinations in Flow Injection Analysis, Analyst, 109 (1984) 413-419.

419. E. Pungor, K. Toth and A. Hrab'czy-Pall, Application of Ion-Selective Electrodes in Flow Analysis, Trends in Anal. Chem., 3 (1984) 28-30.

420. P.W. Hansen, Determination of Fungal α-Amylase by Flow Injection Analysis, Anal. Chim. Acta, 158 (1984) 375-377.

421. C-C. Lee and B.D. Pollard, Determination of Iodine Value of Fatty Acids by a Flow Injection Method, Anal. Chim. Acta, 158 (1984) 157-167.

422. C. Silfwerbrand-Lindh, L. Nord, L.-G. Danielsson and F. Ingman, The Analysis of Aqueous Solutions with Ethanol Soluble Reagents in a Flow Injection System. Spectrophotometric Determination of Uranium, Anal. Chim. Acta, 160 (1984) 11-19.

423. T.P. Lynch, N.J. Kernoghan and J.N. Wilson, Speciation of Metals in Solution by Flow Injection Analysis. Part I: Sequential Spectrophotometric and Atomic-Absorption Detectors, Analyst, 109 (1984) 839-842.

424. T.P. Lynch, N.J. Kernoghan and J.N. Wilson, Speciation of Metals in Solution by Flow Injection Analysis. Part II: Determination of Iron(III) and Iron(II) in Mineral Process Liquors by Simultaneous Injection into Parallel Streams, Analyst, 109 (1984) 843-846.

425. B.F. Rocks, R.A. Sherwood and C. Riley, Controlled-Dispersion Flow Analysis in Clinical Chemistry: Determination of Albumin, Triglycerides and Theophylline, Analyst, 109 (1984) 847-850.

426. A.G. Fogg, A.Y. Chamsi, A.A. Barros and J.O. Cabral, Flow Injection Voltammetric Determination of Hypochlorite and Hypobromite as Bromine by Injection into an Acidic Bromide Eluent and the Indirect Determination of Ammonia and Hydrazine by Reaction with an Excess of Hypobromite, Analyst, 109 (1984) 901-904.

427. R.F. Browner and A.W. Boorn, Sample Introduction: The Ackilles' Heal of Atomic Spectroscopy, Anal. Chem., 56 (1984) 786A-798A.

428. R.F. Browner and A.W. Boorn, Sample Introduction: Technique for Atomic Spectroscopy, Anal. Chem., 56 (1984) 875A-888A.

429. K.M. Korfhage, K. Ravichandran and R.P. Baldwin, Phthalocyanine-Containing Chemically Modified Electrodes for Electrochemical Detection in Liquid Chromatography/Flow Injection Systems, Anal. Chem., 56 (1984) 1514-1517.

430. R. Kuroda, I. Ida and K. Oguma, Determination of Phosphorus in Silicate Rocks by Flow Injection Method of Analysis, Mikrochim. Acta, 1 (1984) 377-383.

431.   T. Yamane, Catalytic Determination of Traces of Cobalt by the Protocatechuic Acid - Hydrogen Peroxide Reaction in a Flow Injection System, Mikrochim. Acta, 1 (1984) 425-433.

432.   J. Wang and H.D. Dewald, Background Current Subtraction in Voltammetric Detection for Flow Injection Analysis, Talanta, 31 (1984) 387-390.

433.   M. Yamada and S. Suzuki, Micellar Enhanced Chemiluminescence of 1,10-Phenanthroline for the Determination of Ultratraces of Copper(II) by Flow Injection Method, Anal. Lett., 17 (1984) 251-263.

434.   P.W. Alexander, M. Trojanowicz and P.R. Haddad, Indirect Potentiometric Determination of Metal Ions by Flow Injection Analysis with a Copper Electrode, Anal. Lett., 17 (1984) 309-320.

435.   F. Malamas, M. Bengtsson and G. Johansson, On-Line Trace Metal Enrichment and Matrix Isolation in Atomic Absorption Spectrometry by a Column Containing Immobilized 8-Quinolinol in a Flow Injection System, Anal. Chim. Acta, 160 (1984) 1-10.

436.   Y. Baba, N. Yoza and S. Ohashi, Simultaneous Determination of Phosphate and Phosphonate by Flow Injection Analysis with a Parallel Detection System, J. Chromatogr., 295 (1984) 153-160.

437.   W.R. Seitz and M.L. Grayeski, Flow-Injection Analysis: A New Approach to Laboratory Automation, J. Clin. Lab. Autom., 4 (1984) 169-182.

438.   M.J. Medina, J. Bartroli, J. Alonso, M. Blanco and J. Fuentes, Direct Determination of Glucose in Blood Serum Using Trinder's Reaction, Anal. Lett., 17 (1984) 385-396.

439.   T. Hara, M. Toriyama and K. Tsukagoshi, Determination of a Small Amount of Biological Constituent by Use of Chemiluminescence. III. The Flow Injection Analysis of Protein by Direct Injection, Bull. Chem. Soc. Jpn., 57 (1984) 1551-1555.

440.   S. Ikeda, H. Satake and Y. Kohri, Flow Injection Analysis with an Amperometric Detector Utilizing the Redox Reaction of Iodate Ion, Chem. Lett., 6 (1984) 873-876.

441.   J.W. Keller, Enzyme Assay by Repetitive Flow Injection Analysis. Application to the Assay of Hog Kidney Aminoacylase, Anal. Lett., 17 (1984) 589-598.

442.   D.S. Austin, J.A. Polta, T.Z. Polta, A.P.C. Tang, T.D. Cabelko and D.C. Johnson, Electrocatalysis at Platinum Electrodes for Anodic Electroanalysis, J. Electroanal. Chem., 168 (1984) 227-248.

443.   H. Wada, G. Nakagawa and K. Ohshita, Synthesis of o,o'-Dihydroxyazo Compounds and Their Application to the Determination of Magnesium and by Flow Injection Analysis, Anal. Chim. Acta, 159 (1984) 289-298.

444.   H. Narasaki and M. Ikeda, Automated Determination of Arsenic and Selenium by Atomic Absorption Spectrometry with Hydride Generation, Anal. Chem., 56 (1984) 2059-2063.

445.   J.C. De Andrade, J.C. Rocha and N. Baccan, Online Oxidation of Chromium(III) to Chromium(VI) for Use with the Flow Injection Analysis Technique, Analyst, 109 (1984) 645-647.

446. S.M. Harden and W.K. Nonidez, Determination of Orthophosphate by Flow Injection Analysis with Amperometric Detection, Anal. Chem., 56 (1984) 2218-2223.

447. M. Bengtsson and G. Johansson, Preconcentration and Matrix Isolation of Heavy Metals through a Two-Stage Solvent Extraction in a Flow System, Anal. Chim. Acta, 158 (1984) 147-156.

448. H.A. Mottola, C.M. Wolff, A. Iob and R. Gnanasekaran, Potentiometric and Amperometric Detection in Flow Injection Enzymatic Determinations, Anal. Chem. Symp. Ser., 18 (1984) 49-75.

449. M. Koupparis, P. Macheras and C. Reppas, Application of Automated Flow Injection Analysis (FIA) to Dissolution Studies, Int. J. Pharm., 20 (1984) 325-333.

450. G.E. Pacey and B.P. Bubnis, Flow Injection Analysis as a Tool for Metal Speciation, Am. Lab., 16 (1984) 26-32.

451. M. Trojanowicz, W. Augustyniak and A. Hulanicki, Photometric Flow-Injection Measurements with a Flow-Cell Employing Light Emitting Diodes, Mikrochim. Acta, 2 (1984) 17-25.

452. P.W. Alexander, P.R. Haddad and M. Trojanowicz, Potentiometric Flow-Injection Determination of Copper-Complexing Inorganic Anions with a Copper-Wire Indicator Electrode, Anal. Chem., 56 (1984) 2417-2422.

453. R.R. Liversage, J.C. Van Loon and J.C. De Andrade, A Flow Injection/Hydride Generation System for the Determination of Arsenic by Inductively-Coupled Plasma Atomic Emission Spectrometry, Anal. Chim. Acta, 161 (1984) 275-283.

454. J.L. Burguera and M. Burguera, Flow Injection Spectrophotometry Followed by Atomic Absorption Spectrometry for the Determination of Iron(II) and Total Iron, Anal. Chim. Acta, 161 (1984) 375-379.

455. N. Yoza, T. Shuto, Y. Baba, A. Tanaka and S. Ohashi, Determination of Complexing Abilities of Ligands for Metal Ions by Flow Injection Analysis and High-Performance Liquid Chromatography, J. Chromatogr., 298 (1984) 419-426.

456. J. Ruzicka and E.H. Hansen, Integrated Microconduits for Flow Injection Analysis, Anal. Chim. Acta, 161 (1984) 1-25.

457. R.C. Schothorst, J.J.F. Van Veen and G. Den Boef, The Application of Strongly Reducing Agents in Flow Injection Analysis. Part 3. Vanadium(II), Anal. Chim. Acta, 161 (1984) 27-35.

458. F.J. Krug, F.O. Bahia and E.A.G. Zagatto, Determination of Molybdenum in Steels by Flow-Injection Spectrophotometry, Anal. Chim. Acta, 161 (1984) 245-255.

459. P. Linares, M.D. Luque de Castro and M. Valcarcel, Spectrofluorometric Flow-Injection Determination of Cyanide, Anal. Chim. Acta, 161 (1984) 257-263.

460. K. Matsumoto, K. Ishida, T. Nomura and Y. Osajima, Conductometric Flow Injection Analysis of the Organic Acid Content in Citrus Fruits, Agric. Biol. Chem., 48 (1984) 2211-2215.

461.   Y. Yang and R.E. Hairell, Single-Laser/Crossed-Beam Thermal Lens Detection for Short Path Length Samples and Flow Injection Analysis, Anal. Chem., 56 (1984) 3002-3004.

462.   R.A. Leach and J.M. Harris, Thermal Lens Absorption Measurements by Flow Injection Into Supercritical Fluid Solvents, Anal. Chem., 56 (1984) 2801-2805.

463.   T.P. Tougas and D.J. Curran, Stopped-Flow Linear Sweep Voltammetry at the Reticulated Vitreous Carbon Electrode in a Flow Injection System. Determinaton of Dopamine in the Presence of Ascorbic Acid, Anal. Chim. Acta, 161 (1984) 325-331.

464.   B.C. Madsen and M.S. Kromis, Flow Injection and Photometric Determination of Hydrogen Peroxide in Rainwater with N-Ethyl-N-(sulfopropyl)aniline Sodium Salt, Anal. Chem., 56 (1984) 2849-2850.

465.   K.G. Schick, High Speed, On-Stream Acid-Base Titration Utilizing Flow Injection Analysis, Adv. Instrum., 39 (1984) 279-285.

466.   A. Rios, M.D. Luque de Castro and M. Valcarcel, Spectrophotometric Determination of Cyanide by Unsegmented Flow Methods, Talanta, 31 (1984) 673-678.

467.   I. Nordin-Andersson, O. Astrom and A. Cedergren, Determination of Water by Flow Injection Analysis with the Karl Fischer Reagent. Minimization of Effects Caused by Differences in Physical Properties of the Samples, Anal. Chim. Acta, 162 (1984) 9-18.

468.   P.J. Worsfold, Clinical Applications of Flow Injection Analysis, Proc. Anal. Div. Chem. Soc., 21 (1984) 376-377.

469.   C. Riley, B.F. Rocks and R.A. Sherwood, Flow-Injection Analysis in Clinical Chemistry, Talanta, 31 (1984) 879-888.

470.   M. Tabata, C. Fukunaga, M. Ohyabu and T. Murachi, Highly Sensitive Flow Injection Analysis of Glucose and Uric Acid in Serum Using an Immobilized Enzyme Column and Chemiluminescence, J. Appl. Biochem, 6 (1984) 251-258.

471.   C.J. Yuan and C.O. Huber, Determination of Proteins and Denaturation Studies by Flow Injection with a Nickel Oxide Electrode, Anal. Chem., 57 (1985) 180-185.

472.   F.F. Cantwell and J.A. Sweileh, Hydrodynamic and Interfacial Origin of Phase Segmentation in Solvent Extraction/Flow Injection Analysis, Anal. Chem., 57 (1985) 329-331.

473.   J.N. Miller, Flow Injection Analysis: Fundamentals and Recent Developments, Proc. Anal. Div. Chem. Soc., 21 (1984) 372-373.

474.   D. Betteridge, A.F. Taylor and A.P. Wade, Optimization of Conditions for Flow Injection Analysis, Proc. Anal. Div. Chem. Soc., 21 (1984) 373-375.

475.   A. Shaw, Practical Aspects of Flow Injection Analysis, Proc. Anal. Div. Chem. Soc., 21 (1984) 375-376.

476.   J.F. Tyson, Flow Injection Analysis Combined with Atomic-Absorption Spectrometry, Proc. Anal. Div. Chem. Soc., 21 (1984) 377-378.

304

477.   M.W. Brown and J. Ruzicka, Parameters Affecting Sensitivity and Precision in the Combination of Flow Injection Analysis with Flame Atomic-Absorption Spectrophotometry, Analyst, 109 (1984) 1091-1094.

478.   S.D. Hartenstein, J. Ruzicka and G.D. Christian, Sensitivity Enhancements for Flow Injection Analysis-Inductively Coupled Plasma Atomic Emission Spectrometry Using an On-Line Preconcentrating Ion-Exchange Column, Anal. Chem., 57 (1985) 21-25.

479.   A.G. Fogg and A.M. Summan, Simple Wall-Jet Cell Holding Either a Solid Electrode or a Sessile Mercury-Drop Electrode and an Illustration of Its Use in the Oxidation and Reduction Flow Injection Voltammetric Determination of Food Coloring Matters, Analyst, 109 (1984) 1029-1034.

480.   S. Honda, T. Konishi and H. Chiba, Evaluation of Dual-Wavelength Spectrophotometry for Drug Level Monitoring, Anal. Chem., 56 (1984) 2352-2354.

481.   N.L. Malavolti, D. Pilosof and T.A. Nieman, Optimization of Experimental Variables for the Chemiluminescent Determination of Glucose in Microporous Membrane Flow Cells, Anal. Chem., 56 (1984) 2191-2195.

482.   J.A. Sweileh and F.F. Cantwell, Sample Introduction by Solvent Extraction/Flow Injection to Eliminate Interferences in Atomic Absorption Spectroscopy, Anal. Chem., 57 (1985) 420-424.

483.   J.A. Polta, I.-H. Yeo and D.C. Johnson, Flow Injection System for the Rapid and Sensitive Assay of Concentrated Aqueous Solutions of Strong Acids and Bases, Anal. Chem., 57 (1985) 563-564.

484.   T. Braun and W.S. Lyon, The Epidemiology of Research on Flow Injection Analysis: An Unconventional Approach, Fresenius' Z. Anal. Chem., 319 (1984) 74-77.

485.   S. Nakashima, M. Yagi, M. Zenki, A. Takahashi and K. Toei, Determination of Nitrate in Natural Waters by Flow Injection Analysis, Fresenius' Z. Anal. Chem., 319 (1984) 506-509.

486.   A. Rios, M.D. Luque de Castro and M. Valcarcel, New Appraoch to the Simultaneous Determination of Pollutants in Waste-Water by FIA. Part I. Anionic Pollutants, Analyst, 109 (1984) 1487-1492.

487.   R.F. Browner, Sample Introduction for Inductively Coupled Plasmas and Flames, Trends in Anal. Chem., 2 (1983) 121-124.

488.   R.C. Schothorst, M. Van Son and G. Den Boef, The Application of Strongly Reducing Agents in Flow Injection Analysis. Part 4. Uranium(III), Anal. Chim. Acta, 162 (1984) 1-8.

489.   P.A. Reardon, G.E. O'Brien and P.E. Sturrock, A Swept-Potential Electrochemical Detector for Flow Streams, Anal. Chim. Acta, 162 (1984) 175-187.

490.   J. Wang and H.D. Dewald, Theoretical and Experimental Aspects of the Response of Stripping Voltammetry in Flow Injection Systems, Anal. Chim. Acta, 162 (1984) 189-196.

491.   K.H. Kroner and M.R. Kula, On-Line Measurement of Extracellular Enzymes During Fermentation by Using Membrane Techniques, Anal. Chim. Acta, 163 (1984) 3-15.

492.   G. Decristoforo and F. Knauseder, Rapid Determination of Cephalosporins with an Immobilized Enzyme Reactor and Sequential Substractive Spectrophotometric Detection in an Automated Flow-Injection System, Anal. Chim. Acta, 163 (1984) 73-84.

493.   C.F. Mandenius, B. Danielsson and B. Mattiasson, Evaluation of a Dialysis Probe for Continuous Sampling in Fermentors and in Complex Media, Anal. Chim. Acta, 163 (1984) 135-141.

494.   F. Winquist, A. Spetz and I. Lundstrom, Determination of Urea with an Ammonia Gas-Sensitive Semiconductor Device in Combination with Urease, Anal. Chim. Acta, 163 (1984) 143-149.

495.   K. Brunt, Rapid Determination of Sulfide in Waste Waters by Continuous Flow Analysis and Gas Diffusion and a Potentiometric Detector, Anal. Chim. Acta, 163 (1984) 293-297.

496.   A. Schelter-Graf, H.L. Schmidt and H. Huck, Determination of the Substrates of Dehydrogenases in Biological Material in Flow-Injection Systems with Electrocatalytic NADH Oxidation, Anal. Chim. Acta, 163 (1984) 299-303.

497.   Z. Fang, J. Ruzicka and E.H. Hansen, An Efficient Flow-Injection System with On-Line Ion-Exchange Preconcentration for the Determination of Trace Amounts of Heavy Metals by Atomic Absorption Spectrometry, Anal. Chim. Acta., 164 (1984) 23-39.

498.   Z. Fang, S. Xu and S. Zhang, The Determination of Trace Amounts of Heavy Metals in Waters by a Flow-Injection System Including Ion-Exchange Preconcentration and Flame Atomic Absorption Spectrometric Detection, Anal. Chim. Acta., 164 (1984) 41-50.

499.   R.A. Leach and J.M. Harris, Real-Time Thermal Lens Absorption Measurements with Application to Flow-Injection Systems, Anal. Chim. Acta, 164 (1984) 91-101.

500.   P.J. Worsfold, J. Farrelly and M.S. Matharu, A Comparison of Spectrophotometric and Chemiluminescence Methods for the Determination of Blood Glucose by Flow Injection Analysis, Anal. Chim. Acta, 164 (1984) 103-109.

501.   F. Winquist, A. Spetz and I. Lundstrom, Determination of Ammonia in Air and Aqueous Samples with a Gas-Sensitive Semiconductor Capacitor, Anal. Chim. Acta, 164 (1984) 127-138.

502.   E. Watanabe, S. Tokimatsu and K. Toyama, Simultaneous Determination of Hypoxanthine, Inosine, Inosine-5'-Phosphate and Adenosine-5'-Phosphate with a Multielectrode Enzyme Sensor, Anal. Chim. Acta, 164 (1984) 139-146.

503.   S. Alegret, J. Alonso, J. Bartroli, J.M. Paulis, J.L.F.C. Lima and A.A.S.C. Machado, Flow-through Tubular PVC Matrix Membrane Electrode without Inner Reference Solution for Flow Injection Analysis, Anal. Chim. Acta, 164 (1984) 147-152.

504. L. Nord and B. Karlberg, Extraction Based on the Flow-Injection Principle. Part 6. Film Formation and Dispersion in Liquid-Liquid Segmented Flow Extraction Systems, Anal. Chim. Acta, 164 (1984) 233-249.

505. F. Lazaro, M.D. Luque de Castro and M. Valcarcel, Stopped-Flow Injection Determination of Copper(II) at the ng/ml Level, Anal. Chim. Acta, 165 (1984) 177-185.

506. A. Fernandez, M.A. Gomez-Nieto, M.D. Luque de Castro and M. Valcarcel, A Flow-Injection Manifold Based on Splitting the Sample Zone and a Confluence Point Before a Single Detector Unit, Anal. Chim. Acta, 165 (1984) 217-226.

507. D. Betteridge, C.Z. Marczewski and A.P. Wade, A Random Walk Simulation of Flow Injection Analysis, Anal. Chim. Acta, 165 (1984) 227-236.

508. M. Masoom and A. Townshend, Determination of Glucose in Blood by Flow Injection Analysis and an Immobilized Glucose Oxidase Column, Anal. Chim. Acta, 166 (1984) 111-118.

509. P.W. Alexander and U. Akapongkul, Differential Pulse Voltammetry with Fast Pulse Repetition Times in a Flow-Injection System with a Copper-Amalgam Electrode, Anal. Chim. Acta, 166 (1984) 119-127.

510. H.D. Dewald and J. Wang, Spectroelectrochemical Detector for Flow-Injection Systems and Liquid Chromatography, Anal. Chim. Acta, 166 (1984) 163-170.

511. R.M. Smith and T.G. Hurdley, Spectrophotometric Determination of Copper as a Dithiocarbamate by Flow Injection Analysis, Anal. Chim. Acta, 166 (1984) 271-276.

512. I. Schneider, Determination of Penicillin V in Fermentation Samples by Flow Injection Analysis, Anal. Chim. Acta, 166 (1984) 293-295.

513. F. Lazaro, M.D. Luque de Castro and M. Valcarcel, Simultaneous Catalytic-Fluorometric Determination of Copper and Mercury by Flow-Injection Analysis, Fresenius' Z. Anal. Chem., 320 (1985) 128-132.

514. J.C. De Andrade, J.C. Rocha and N. Baccan, Sequential Spectrophotometric Determination of Chromium(III) and Chromium(VI) Using Flow Injection Analysis, Analyst, 110 (1985) 197-199.

515. O.K. Borggaard and S.S. Jorgensen, Determination of Silicon in Soil Extracts by Flow Injection Analysis, Analyst, 110 (1985) 177-180.

516. M.G. Glaister, G.J. Moody and J.D.R. Thomas, Studies on Flow Injection Analysis with Sulphide Ion-Selective Electrodes, Analyst, 110 (1985) 113-119.

517. T. Greatorex and P.B. Smith, Flow Injection Analysis - A Review of Experiences in a Water Authority Laboratory, J. Inst. Water Eng. Scient., 39 (1985) 81-91.

518. M.O. Babiker and J.A.W. Dalziel, Studies on the Determination of Sulphide Using N,N-Diethyl-p-Phenylenediamine, Anal. Proc. Div. Chem. Soc., 20 (1983) 609-611.

519. D.S. Polcyn, Literature Review: Chemical Analysis of Water and Waste-Water. Inorganics, J. Water Pollut. Control Fed., 56 (1984) 502-522.

520. P.C. Thijssen, G. Kateman and H.C. Smith, State Estimation as Chemometrical System, Trends in Anal. Chem., 4 (1985) 70-74.

521. A. Rios, M.D. Luque de Castro and M. Valcarcel, New Approach to the Simultaneous Determination of Pollutants in Waste Water by Flow Injection Analysis. Part 2. Cationic Pollutants, Analyst, 110 (1985) 277-281.

522. K.G. Miller, G.E. Pacey and G. Gordon, Automated Iodometric Method for the Determination of Trace Chlorate Ion Using Flow Injection Analysis, Anal. Chem., 57 (1985) 734-737.

523. M.A. Marshall and H.A. Mottola, Performance Studies Under Flow Conditions of Silica Immobilized 8-Quinolinol and Its Application as a Preconcentration Tool in Flow Injection/Atomic Absorption Determinations, Anal. Chem., 57 (1985) 729-733.

524. J. Moller and B. Winter, Application of Flow Injection Techniques for the Analysis of Inorganic Anions, Fresenius' Z. Anal. Chem., 320 (1985) 451-456.

525. A.M.G. Macdonald and T.A. Nieman, Flow Injection and Liquid Chromatography Detector for Amino Acid Based on a Postcolumn Reaction with Luminol, Anal. Chem., 57 (1985) 936-940.

526. L. Fossey and F.F. Cantwell, Determination of Acidity Constants by Solvent Extraction/Flow Injection Analysis Using a Dual-Membrane Phase Separator, Anal. Chem., 57 (1985) 922-926.

527. T. Yao, M. Sato, Y. Kobayashi and T. Wasa, Flow Injection Analysis for Glucose by the Combined Use of an Immobilized Glucose Oxidase Reactor and a Peroxidase Electrode, Anal. Chim. Acta., 165 (1984) 291-296.

528. J.J. Harrow, J. Janata, R.L. Stephen and W.J. Kolff, Portable System for Simultaneous Measurements of Blood Electrolytes, Proc. EDTA, 17 (1980) 179-187.

529. E. Martins and G. Johansson, Solute Transfer in On-Line Analytical Flow-through Dialyzers, Anal. Chim. Acta, 167 (1985) 111-122.

530. B. Olsson, H. Lundback and G. Johansson, Galactose Determination in an Automated Flow-Injection System Containing Enzyme Reactors and an On-Line Dialyzer, Anal. Chim. Acta, 167 (1985) 123-136.

531. A.T. Faizullah and A. Townshend, Application of a Reducing Column for Metal Speciation by Flow Injection Analysis, Anal. Chim. Acta, 167 (1985) 225-231.

532. M. Maeda and A. Tsuji, Enzymatic Immunoassay of Alpha-Fetoprotein, Insulin and 17-Alpha-Hydroxyprogesteron Based on Chemiluminescence in a Flow-Injection System, Anal. Chim. Acta, 167 (1985) 241-248.

533. P. Van Zoonen, D.A. Kamminga, C. Gooijer, N.H. Velthorst and R.W. Frei, Flow Injection Determination of Hydrogen Peroxide by Means of a Solid-State Peroxyoxalate Chemiluminescence Reactor, Anal. Chim. Acta, 167 (1985) 249-256.

534. Y. Hirai and K. Tomokuni, Extraction-Spectrophotometric Determination of Anionic Surfactants with a Flow-Injection System, Anal. Chim. Acta, 167 (1985) 409-412.

535.   A.G. Fogg, M.A. Fernandez-Arciniega and R.M. Alonso, Flow Injection Amperometric Determination of Nitroprusside at a Glassy Carbon Electrode and at a Sessile Mercury Drop Electrode, Analyst, 110 (1985) 345-348.

536.   A.G. Fogg, A.M. Summan and M.A. Fernandez-Arciniega, Flow Injection Amperometric Determination of Ascorbic Acid and Dopamine at a Sessile Mercury Drop Electrode without Deoxygenation, Analyst, 110 (1985) 341-343.

537.   A.G. Cox, I.G. Cook and C.W. McLeod, Rapid Sequential Determination of Chromium(III) - Chromium(VI) by Flow Injection Analysis - Inductively Coupled Plasma Atomic-Emission Spectrometry, Analyst, 110 (1985) 331-333.

538.   S. Tanabe, T. Shioiri, K. Murakami and T. Imanari, A New Method for Assay of Ferroxidase Activity and Its Application to Human and Rabbit Serums, Chem. Pharm. Bull., 32 (1984) 4029-4035.

539.   M. Herrera, L.-S. Kao, D.J. Curran and E.W. Westhead, Flow Injection Analysis of Catecholamine Secretion from Bovine Adrenal Medulla Cells on Microbeads, Anal. Biochem., 144 (1985) 218-227.

540.   M. Miyazaki, N. Okubo, K. Hayakawa and T. Umeda, Specific and Selective Determination Method for Halide Anions by a Flow Injection Technique, Chem. Pharm. Bull, 32 (1984) 3702-3705.

541.   K. Hult, R. Fuchs, M. Peraica, R. Plestina and S. Ceovic, Screening for Ochratoxin A in Blood by Flow Injection Analysis, J. Appl. Toxicol., 4 (1984) 326-329.

542.   C. Gooijer, N.H. Velthorst and R.W. Frei, Phosphorescence in Liquid Solutions: A Promising Detection Principle in Liquid Chromatography and Flow Injection Analysis, Trends in Anal. Chem., 3 (1984) 259-265.

543.   S.A. McClintok, J.R. Weber and W.C. Purdy, The Design of a Computer-Controlled Flow-Injection Analyzer: An Undergraduate Experiment, J. Chem. Educ., 62 (1985) 65-67.

544.   Z. Niegreisz, L. Szucs, J. Fekete, G. Horvai, K. Toth and E. Pungor, Modifications of the Wall-Jet Electrochemical Detector for Liquid Chromatography and Flow Analysis, J. Chromatogr., 316 (1984) 451-459.

545.   J.B. Landis, Flow Injection Analysis in Pharmaceutical Analysis, Drugs Pharm. Sci., 11 (1984) 217-277.

546.   Y.P. Wu and G.E. Pacey, Spectrophotometric Determination of Lithium Ion with the Chromogenic Crown Ether, 2",4"-Dinitro-6"-Trifluoromethylphenyl 4'-Aminobenzo-14-Crown-4, Anal. Chim. Acta, 162 (1984) 285-291.

547.   M. Masoom and A. Townshend, Applications of Immobilized Enzymes in Flow Injection Analysis, Proc. Anal. Div. Chem. Soc., 22 (1985) 6-8.

548.   M.H. Ho and M.U. Asouzu, Use of Immobilized Enzymes in Flow Injection Analysis, Ann. N. Y. Acad. Sci., 434 (1984) 526.

549.   Y. Baba, N. Yoza and S. Ohashi, Simultaneous Determination of Phosphate and Phosphonates by Flow Injection Analysis and High-Performance Liquid Chromatography with a Series Detection System, J. Chromatogr., 318 (1985) 319-321.

550. A.T. Faizullah and A. Townshend, Flow Injection Analysis with Chemiluminescence Detection: Determination of Hydrazine, Proc. Anal. Div. Chem. Soc., 22 (1985) 15-16.

551. J.F. Tyson and J.M.H. Appleton, Concentration Gradients for Calibration Purposes, Proc. Anal. Div. Chem. Soc., 22 (1985) 17-19.

552. A. Hughes and P.J. Worsfold, Monitoring of Immunoprecipitin Reactions Using Flow Injection Analysis, Proc. Anal. Div. Chem. Soc., 22 (1985) 16-17.

553. P. Linares, M.D. Luque de Castro and M. Valcarcel, Fluorometric Determination of Pyridoxal and Pyridoxal-5-Phosphate by Flow Injection Analysis, Anal. Lett.(B), 18 (1985) 67-78.

554. R. Gnanasekaran and H.A. Mottola, Flow Injection Determination of Penicillins Using Immobilized Penicillinase in a Single Bead String Reactor, Anal. Chem., 57 (1985) 1005-1009.

555. K. Fujiwara and K. Fuwa, Liquid Core Optical Fiber Total Reflection Cell as a Colorimetric Detector for Flow Injection Analysis, Anal. Chem., 57 (1985) 1012-1016.

556. P.K. Dasgupta and H. Hwang, Application of a Nested Loop System for the Flow Injection Analysis of Trace Aqueous Peroxides, Anal. Chem., 57 (1985) 1009-1012.

557. M.H. Ho, Microprocessor-Controlled Flow Injection Analyzer for Biochemical Applications, Biomed. Sci. Instr., 20 (1984) 93-97.

558. M.A. Koupparis and P.I. Anagnostopoulou, An Automated Microprocessor-Based Spectrophotometric Flow-Injection Analyzer, J. Autom. Chem., 6 (1984) 186-191.

559. A. Recktenwald, K.H. Kroner and M.-R. Kula, Rapid On-Line Protein Detection in Biotechnological Processes by Flow Injection Analysis, Enzyme Microb. Technol., 7 (1985) 146-149.

560. F. Lazaro, M.D. Luque de Castro and M. Valcarcel, Flow Injection Environmental Analysis: A Review, Analusis, 13 (1985) 147-159.

561. T.P. Tougas, J.M. Janetti and W.G. Collier, Theoretical and Experimental Response of a Biamperometric Detector for Flow Injection Analysis, Anal. Chem., 57 (1985) 1377-1381.

562. J.E. Newbery and M.P. Lopez de Haddad, Amperometric Determination of Nitrite by Oxidation at a Glassy Carbon Electrode, Analyst, 110 (1985) 81-82.

563. J.A. Polta and D.C. Johnson, Pulsed Amperometric Detection of Electroinactive Adsorbates at Platinum Electrodes in a Flow Injection System, Anal. Chem., 57 (1985) 1373-1376.

564. C.C.Y. Chan, Semiautomated Method for the Determination of Selenium in Geological Materials Using a Flow Injection Analysis Technique, Anal. Chem., 57 (1985) 1482-1485.

565. M. Yamamoto, M. Yasuda and Y. Yamamoto, Hydride-Generation Atomic Absorption Spectrometry Coupled with Flow Injection Analysis, Anal. Chem., 57 (1985) 1382-1385.

310

566. Z. Fang, J.M. Harris, J. Ruzicka and E.H. Hansen, Simultaneous Flame Photometric Determination of Lithium, Sodium, Potassium, and Calcium by Flow Injection Analysis with Gradient Scanning Standard Addition, Anal. Chem., 57 (1985) 1457-1461.

567. C.W. McLeod, I.G. Cook, P.J. Worsfold, J.E. Davies and J. Queay, Analyte Enrichment and Matrix Removal in Flow Injection Analysis-Inductively Coupled Plasma-Atomic Emission Spectrometry: Determination of Phosphorus in Steels, Spectrochim. Acta, 40B (1985) 57-62.

568. T. Hara, M. Toriyama, T. Ebuchi and M. Imaki, Flow Injection Analysis of Alpha-Amino Acids by the Chemiluminescence Method, Chem. Lett., 3 (1985) 341-342.

569. K.S. Johnson, R.L. Petty and J. Thomsen, Flow-Injection Analysis for Seawater Micronutrients, Adv. Chem. Ser., 209 (1985) 7-30

570. T. Toyoda, S.S. Kuan and G.G. Guilbault, Determination of Lactate Dehydrogenase Isoenzyme (LD-1) Using Flow Injection Analysis with Electrochemical Detection after Immunochemical Separation, Anal. Lett., 18B (1985) 345-355.

571. K. Kurkijarvi, T. Heinonen, T. Lovgren, J. Lavi and R. Raunio, Flow-Injection Analysis with Immobilized Bacterial Bioluminescence Enzymes, Anal. Appl. Biolumin. Chemilumin. (Proc. Int. Symp.), 3 (1984) 125-128.

572. C. Hongbo, E.H. Hansen and J. Ruzicka, Evaluation of Critical Parameters for Measurement of pH by Flow Injection Analysis, Anal. Chim. Acta, 169 (1985) 209-220.

573. L.T.M. Prop, P.C. Thijssen and L.G.G. Van Dongen, A Software Package for Computer-Controlled Flow-Injection Analysis, Talanta, 32 (1985) 230-234.

574. J.T. Shih and P.W. Carr, Flow-Rate Dependence of Post-Column Reaction Chromatographic Detectors and Optimization of Reactor Length for Slow Chemical Reactions, Anal. Chim. Acta, 167 (1985) 137-144.

575. M. Yamada, H. Kanai and S. Suzuki, Flavin Mononucleotide Chemiluminescence for Determination of Traces of Copper(II) by Continuous Flow and Flow Injection Methods, Bull. Chem. Soc. Jap., 58 (1985) 1137-1142.

576. E.B. Milosavljevic, J. Ruzicka and E.H. Hansen, Simultaneous Determination of Free and EDTA-Complexed Copper Ions by Flame Atomic Absorption Spectrometry with an Ion-Exchange Flow Injection System, Anal. Chim. Acta, 169 (1985) 321-324.

577. J. Wang and L.D. Hutchins, Thin-Layer Electrochemical Detector with a Glassy Carbon Electrode Coated with a Base-Hydrolysed Cellulosic Film, Anal. Chem., 57 (1985) 1536-1541.

578. M.A. Gomez-Nieto, M.D. Luque de Castro, A. Martin and M. Valcarcel, Prediction of the Behaviour of a Single Flow-Injection Manifold, Talanta, 32 (1985) 319-324.

579. J.T. Vanderslice and G.R. Beecher, Comments on the Paper by Gomez-Nieto, Luque de Castro, Martin and Valcarcel, Talanta, 32 (1985) 334-335.

580.   M. Valcarcel and M.D. Luque de Castro, Reply to the Comments by Vanderslice and Beecher, Talanta, 32 (1985) 339-340.

581.   R.C. Schothorst and G. Den Boef, The Application of Strongly Oxidizing Agents in Flow Analysis. Part I: Silver(II), Anal. Chim. Acta, 169 (1985) 99-107.

582.   R. Kuroda, I. Ida and H. Kimura, Spectrophotometric Determination of Silicon in Silicates by Flow-Injection Analysis, Talanta, 32 (1985) 353-357.

583.   A. Rios, M.D. Luque de Castro and M. Valcarcel, Flow Injection Analysis: A New Approach to Pharmaceutical Determinations, J. Pharm. Biomed. Anal., 3 (1985) 105-121.

584.   J.L. Burguera and M. Burguera, The Principles, Applications and Trends of Flow Injection Analysis for Monitoring Chemiluminescence Reactions, Acta Cient. Venez., 34 (1983) 79-83.

585.   S.S. Jorgensen, K.M. Petersen and L.A. Hansen, A Simple Multifunctional Valve for Flow Injection Analysis, Anal. Chim. Acta, 169 (1985) 51-57.

586.   E.A. Jones, Spectrophotometric Determination of Uranium (VI) with 2-(5-Bromo-2-Pyridylazo)-5-Diethylaminophenol in a Flow-Injection System, Anal. Chim. Acta, 169 (1985) 109-115.

587.   J.M. Harnly and G.R. Beecher, Two-Valve Injector to Minimize Nebulizer Memory for Flow Injection Atomic Absorption Spectrometry, Anal. Chem., 57 (1985) 2015-2016.

588.   M.R. Straka, G. Gordon and G.E. Pacey, Residual Aqueous Ozone Determination by Gas Diffusion Flow Injection Analysis, Anal. Chem., 57 (1985) 1799-1803.

589.   H. Hwang and P.K. Dasgupta, Fluorometric Determination of Trace Hydrogen Peroxide in Water with a Flow Injection System, Anal. Chim. Acta, 170 (1985) 347-351.

590.   J.M. Hungerford, G.D. Christian, J. Ruzicka and J.C. Giddings, Reaction Rate Measurement by Flow Injection Analysis Using a Gradient Stopped-Flow Method, Anal. Chem., 57 (1985) 1794-1798.

591.   R.S. Schifreen, Flow Enthalpimetry (Isoperibol Flow-Injection Calorimetry), Chem. Anal., 79 (1985) 97-135.

592.   A. Rios, M.D. Luque de Castro and M. Valcarcel, Multidetection in Unsegmented Flow Systems with a Single Detector, Anal. Chem., 57 (1985) 1803-1805.

593.   S.D. Rothwell and A.A. Woolf, A Timed Solenoid Injector for Flow Analysis, Talanta, 32 (1985) 431-433.

594.   M.A. Koupparis and P.I. Anagnostopoulou, Automated Flow-Injection Pseudotitration of Strong and Weak Acids, Ascorbic Acid and Calcium, and Catalytic Pseudotitrations of Aminopolycarboxylic Acids, Talanta, 32 (1985) 411-417.

595.   F. Lazaro, M.D. Luque de Castro and M. Valcarcel, Sequential and Differential Catalytic-Fluorimetric Determination of Manganese and Iron by Flow Injection Analysis, Anal. Chim. Acta, 169 (1985) 141-148.

596. W. LaCourse and I.S. Krull, Photoelectrochemical Detector for High Performance Liquid Chromatography and Flow Injection Analysis, Anal. Chem., 57 (1985) 1810-1814.

597. H. Huck, A. Schelter-Graf and H.L. Schmidt, Measurement and Calculation of the Calibration Graphs for Flow Injection Analysis Using Enzyme Reactors with Immobilized Dehydrogenases and an Amperometric NADH Detector, Bioelectrochem. Bioenerg., 13 (1984) 199-209.

598. A.J. Faske, K.R. Snable, A.W. Boorn and R.F. Browner, Microliter Sample Introduction for ICP-AES, Appl. Spectrosc., 39 (1985) 542-545.

599. M. Gallego and M. Valcarcel, Indirect Atomic Absorption Spectrometric Determination of Perchlorate by Liquid-Liquid Extraction in a Flow-Injection System, Anal. Chim. Acta, 169 (1985) 161-169.

600. R.A. Mowery, The Potential of Flow-Injection Techniques for On-Stream Process Applications, ISA Trans., 24 (1985) 1-9.

601. J.F. Tyson, Flow Injection Techniques for Flame Atomic Absorption Spectrophotometry, Trends in Anal. Chem., 4 (1985) 124-128.

602. N. Yoza, H. Hirano, Y. Baba and S. Ohashi, Characterization of Enzymatic Hydrolysis of Inorganic Polyphosphates by Flow Injection Analysis and High-Performance Liquid Chromatography, J. Chromatogr., 325 (1985) 385-393.

603. P. Martinez-Jimenez, M. Gallego and M. Valcarcel, Indirect Atomic Absorption Determination of Uranium by Flow Injection Analysis Using an Air-Acetylene Flame, At. Spectrosc., 6 (1985) 65-68.

604. A. Tanaka, M. Miyazaki and T. Deguchi, New Simultaneous Catalytic Determination of Thiocyanate and Iodide by Flow Injection Analysis, Anal. Lett., 18 (1985) 695-705.

605. F. Lazaro, M.D. Luque de Castro and M. Valcarcel, Catalytic-Fluorimetric Determination of EDTA and Iron(III) by Flow Injection Analysis. Inhibition Methods, Fresenius' Z. Anal. Chem., 321 (1985) 467-470.

606. P. Linares, M.D. Luque de Castro and M. Valcarcel, Simultaneous Determination of Pyridoxal and Pyridoxal-5-Phosphate in Human Serum by Flow Injection Analysis, Anal. Chem., 57 (1985) 2101-2106.

607. E. Martins, M. Bengtsson and G. Johansson, On-Line Dialysis of Some Metal Ions and Metal Complexes, Anal. Chim. Acta, 169 (1985) 31-42.

608. K. Backstrom, L.-G. Danielsson and L. Nord, Design and Evaluation of a New Phase Separator for Liquid-Liquid Extraction in Flow Systems, Anal. Chim. Acta, 169 (1985) 43-49.

609. R. Appelquist, G. Marko-Varga, L. Gorton, A. Torstensson and G. Johansson, Enzymatic Determination of Glucose in a Flow System by Catalytic Oxidation of the Nicotinamide Coenzyme at a Modified Electrode, Anal. Chim. Acta, 169 (1985) 237-247.

610. M.L. Hitchman and F.W.M. Nyasulu, Indirect Potentiometric Monitoring of Proteins with a Copper Electrode, Anal. Chim. Acta, 173 (1985) 337-342.

611. H. Husted, K.H. Kroner and M.-R. Kula, Process Control in Enzyme Production and Purification by On-Line Dialysis, BTF - Biotech-Forum, 2 (1985) 56-57.

612. J.L. Burguera, M. Burguera and D. Flores, Determination of Some Phosphorus-Containing Compounds by Flow Injection with a Molecular Emission Cavity Detector, Anal. Chim. Acta, 170 (1985) 331-336.

613. N.L. Malavolti, D. Pilosof and T.A. Nieman, Determination of Cholesterol with a Microporous Membrane Chemiluminescence Cell with Cholesterol Oxidase in Solution, Anal. Chim. Acta, 170 (1985) 199-207.

614. P.W. Alexander, P.R. Haddad and M. Trojanowicz, Potentiometric Flow-Injection Determination of Copper-Complexing Organic Ligands with a Copper-Wire Indicating Electrode, Anal. Chim. Acta, 171 (1985) 151-163.

615. M. Masoom and A. Townshend, Simultaneous Determination of Sucrose and Glucose in Mixtures by Flow Injection Analysis with Immobilized Enzymes, Anal. Chim. Acta, 171 (1985) 185-192.

616. A. Rios, M.D. Luque de Castro and M. Valcarcel, Spectrophotometric Determination of Acidity-Constants of Unstable Compounds by Flow Injection Analysis, Anal. Chim. Acta, 171 (1985) 303-312.

617. P.J. Worsfold and A. Nabi, The Bioluminescent Determination of Adenosine Triphosphate with a Flow-Injection System, Anal. Chim. Acta, 171 (1985) 333-336.

618. M.C.U. Araujo, C. Pasquini, R.E. Bruns and E.A.G. Zagatto, A Fast Procedure for Standard Additions in Flow Injection Analysis, Anal. Chim. Acta, 171 (1985) 337-343.

619. H. Lundback and B. Olsson, Amperometric Determination of Galactose, Lactose and Dihydroxyacetone Using Galactose Oxidase in a Flow Injection System with Immobilized Enzyme Reactors and On-Line Dialysis, Anal. Lett., 18B (1985) 871-889.

620. T. Yao, M. Sato, Y. Kobayashi and T. Wasa, Amperometric Assay of Total and Free Cholesterols in Serum by the Combined Use of Immobilized Cholesterol Esterase and Cholesterol Oxidase Reactors and Peroxidase Electrode, Anal. Biochem., 149 (1985) 387-391.

621. K. Matsumoto, O. Hamada, H. Ukeda and Y. Osajima, Flow Injection Analysis of Lactose in Milk Using a Chemically Modified Lactose Electrode, Agric. Biol. Chem., 49 (1985) 2131-2135.

622. J.F. Van Staden, Automated Prevalve Dilution in Flow-Injection Analysis. The Automated Determination of Chloride in Surface, Ground and Domestic Water, Fresenius' Z. Anal. Chem., 322 (1985) 36-41.

623. G. Schulze, W. Bonigk and W. Frenzel, Matrix Exchange Techniques for the Simultaneous Determination of Several Elements in Flow Injection Potentiometric Stripping Analysis, Fresenius' Z. Anal. Chem., 322 (1985) 255-260.

624. L. Ilcheva and K. Cammann, Flow Injection Analysis of Chloride in Tap and Sewage Water Using Ion-Selective Electrode Detection, Fresenius' Z. Anal. Chem., 322 (1985) 323-326.

625.   J. Ruz, A. Rios, M.D. Luque de Castro and M. Valcarcel, Simultaneous and Sequential Determination of Chromium(VI) and Chromium(III) by Unsegmented Flow Methods, Fresenius' Z. Anal. Chem., 322 (1985) 499-502.

626.   K.L. Jansen and J.M. Harris, Double Beam Thermal Lens Spectrometry, Anal. Chem., 57 (1985) 2434-2436.

627.   I. Nordin-Andersson and A. Cedergren, Spectrophotometric Determination of Water by Flow Injection Analysis Using Conventional and Pyridine-Free Two-Component Karl-Fischer Reagents, Anal. Chem., 57 (1985) 2571-2575.

628.   D.A. Hollowell, G.E. Pacey and G. Gordon, Selective Determination of Chlorine Dioxide Using Gas Diffusion Flow Injection Analysis, Anal. Chem., 57 (1985) 2851-2854.

629.   D.K. Cope and D.E. Tallman, Calculation of Convective Diffusion Current at a Strip Electrode in a Rectangular Flow Channel: Inviscid Flow, J. Electroanal. Chem. Interfacial Electrochem., 188 (1985) 21-31.

630.   J.A. Olivares and R.S. Houk, Ion Sampling for Inductively Coupled Plasma Mass Spectrometry, Anal. Chem., 57 (1985) 2674-2679.

631.   A.F. Gomez, J.R. Polonio, M.D. Luque de Castro and M. Valcarcel, Automated Enzymatic-Fluorimetric Determination of Ethanol in Blood by Flow Injection Analysis, Clin. Chim. Acta, 148 (1985) 131-137.

632.   J.A. Sweileh and F.F. Cantwell, Use of Peak Height for Quantification in Solvent Extraction/Flow Injection Analysis, Can. J. Chem., 63 (1985) 2559-2563.

633.   M. Maeda and A. Tsuji, Fluorescence and Chemiluminescence Determination of Steroid and Bile Acid Sulphates with Lucigenin by Flow Injection Analysis Based on Ion-Pair Solvent Extraction, Analyst, 110 (1985) 665-668.

634.   J.F. Tyson, Flow Injection Analysis Techniques for Atomic-Absorption Spectrometry, Analyst, 110 (1985) 419-429.

635.   R.A. Sherwood, B.F. Rocks and C. Riley, Controlled-Dispersion Flow Analysis with Atomic-Absorption Detection for the Determination of Clinically Relevant Elements, Analyst, 110 (1985) 493-496.

636.   B.F. Rocks, S.M. Wartel, R.A. Sherwood and C. Riley, Determination of Albumin with Bromocresol Purple Using Controlled-Dispersion Flow Analysis, Analyst, 110 (1985) 669-671.

637.   R.A.E. Escott and A.F. Taylor, Determination of Water by Flow Injection Analysis Using Karl Fischer Reagent with Electrochemical Detection, Analyst, 110 (1985) 847-849.

638.   A.G. Fogg, M.A. Fernandez-Arciniega and R.M. Alonso, Oxidative Amperometric Flow Injection Determination of Sulphite at an Electrochemically Pre-Treated Glassy Carbon Electrode, Analyst, 110 (1985) 851-853.

639.   P.I. Anagnostopoulou and M.A. Koupparis, Automated Flow-Injection Pseudotitration of Boric Acid, J. Pharm. Sci., 74 (1985) 886-888.

640.   M. Masoom and A. Townshend, Determination of Cholesterol by Flow Injection Analysis with Immobilized Cholesterol Oxidase, Anal. Chim. Acta, 174 (1985) 293-297.

641.   J.J. Harrow and J. Janata, Heterogeneous Samples in Flow-Injection Systems. Part 1: Whole Blood, Anal. Chim. Acta, 174 (1985) 115-122.

642.   J.J. Harrow and J. Janata, Heterogenous Samples in Flow-Injection Systems. Part 2: Standard Addition, Anal. Chim. Acta, 174 (1985) 123-132.

643.   J.A. Wise, W.R. Heineman and P.T. Kissinger, Flow Injection System for Stripping Voltammetry, Anal. Chim. Acta, 172 (1985) 1-12.

644.   A.T. Faizullah and A. Townshend, Spectrophotometric Determination of Copper by Flow Injection Analysis with an On-Line Reduction Column, Anal. Chim. Acta, 172 (1985) 291-296.

645.   H. Wada, H. Mori and G. Nakagawa, Spectrophotometric Determination of Fluoride with Lanthanum/Alizarin Complexone by Flow Injection Analysis, Anal. Chim. Acta, 172 (1985) 297-302.

646.   J. Ruzicka and E.H. Hansen, Optosensing of Active Surfaces - A New Detection Principle in Flow Injection Analysis, Anal. Chim. Acta, 173 (1985) 3-21.

647.   E.A.G. Zagatto, M.F. Gine, E.A.N. Fernandes, B.F. Reis and F.J. Krug, Sequential Injections in Flow Systems as an Alternative to Gradient Exploatation, Anal. Chim. Acta, 173 (1985) 289-297.

648.   J. Kurzawa, Determination of Sulphur(II) Compounds by Flow Injection Analysis with Application of the Induced Iodine/Azide Reaction, Anal. Chim. Acta, 173 (1985) 343-348.

649.   J.T. Dyke and Q. Fernando, Deconvolution Techniques for Rapid Flow-Injection Analysis, Talanta, 32 (1985) 807-810.

650.   A. Rios, M.D. Luque de Castro and M. Valcarcel, Injection Analysis with Flow-Gradient Systems: A New Approach to Unsegmented Flow Techniques, Talanta, 32 (1985) 845-850.

651.   R.A. Salerno, C. Odell, N. Cyanovich, B.P. Bubnis, W. Morges and A. Gray, Lowry Protein Determination by Automated Flow Injection Analysis for Bovine Serum Albumin and Hepatitis B Surface Antigen, Anal. Biochem., 151 (1985) 309-314.

652.   K. Toei, T. Zaitsu and C. Igarashi, Spectrophotometric Determination of Micro Amounts of Cationic Polymeric Flocculants by Flow Injection Analysis, Anal. Chim. Acta, 174 (1985) 369-373.

653.   M.J. Whitaker, Determination of Total Chromium by Flow Injection Analysis, Anal. Chim. Acta, 174 (1985) 375-378.

654.   P.C. Thijssen, L.T.M. Prop, G. Kateman and H.C. Smit, A Kalman Filter for Calibration, Evaluation of Unknown Samples and Quality Control in Drifting Systems. Part 4. Flow Injection Analysis, Anal. Chim. Acta, 174 (1985) 27-40.

316

655.   F. Lazaro, M.D. Luque de Castro and M. Valcarcel, Catalytic-Fluorometric Determination of Titanium(IV) by Flow Injection Analysis, Anal. Lett., 18A (1985) 1209-1220.

656.   D. Betteridge, A.P. Wade and A.G. Howard, Reflections on the Modified Simplex-I, Talanta, 32 (1985) 709-722.

657.   D. Betteridge, A.P. Wade and A.G. Howard, Reflections on the Modified Simplex-II, Talanta, 32 (1985) 723-734.

658.   P. Martinez-Jimenez, M. Gallego and M. Valcarcel, Indirect Atomic Absorption Determination of Cerium and Lanthanum by Flow Injection Analysis Using an Air-Acetylene Flame, At. Spectrosc., 6 (1985) 137-141.

659.   H. Casey and S. Smith, Application of Flow Injection Analysis to Water Pollution Studies, Trends in Anal. Chem., 4 (1985) 256-258.

660.   C. Wechter, N. Sleszynski, J.J. O'Dea and J. Osteryoung, Anodic Stripping Voltammetry with Flow Injection Analysis, Anal. Chim. Acta, 175 (1985) 45-53.

661.   S. Johansson, H. Brotell and L. Nord, Flow-Injection Extraction and Gas-Chromatographic Determination of Terodiline in Blood Serum, Anal. Chim. Acta, 175 (1985) 281-287.

662.   T. Yao and T. Wasa, Simultaneous Determination of L(+)- and D(-)-Lactic Acid By Use of Immobilized Enzymes in a Flow-Injection System, Anal. Chim. Acta, 175 (1985) 301-304.

663.   R.C. Schothorst and G. Den Boef, The Application of Strongly Reducing Agents in Flow Injection Analysis. Part 5. Chromium(II) and Vanadium(II) in Acidic Medium, Anal. Chim. Acta, 175 (1985) 305-308.

664.   T. Kato, Flow-Injection Spectrophotometric Determination of Enalapril in Pharmaceuticals with Bromothymol Blue, Anal. Chim. Acta, 175 (1985) 339-344.

665.   A.G. Fogg, M.A. Fernandez-Arciniega and R.M. Alonso, Amperometric Flow Injection Determination of Ethylendiaminetetraacetic Acid (EDTA) at an Electrochemically Pre-Treated Glassy Carbon Electrode, Analyst, 110 (1985) 1201-1204.

666.   G.L. Abdullahi and J.N. Miller, Application of Gradient Flow Injection Analysis to Studies of Drug-Protein Binding, Analyst, 110 (1985) 1271-1272.

667.   P.J. Worsfold, A. Hughes and D.J. Mowthorpe, Determination of Human Serum Immunoglobulin G Using Flow Injection Analysis with Rate Turbidimetric Detection, Analyst, 110 (1985) 1303-1305.

668.   A. Recktenwald, K.H. Kroner and M.-R. Kula, On-Line Monitoring of Enzymes in Downstreams Processing by Flow Injection Analysis (FIA), Enzyme Microb. Technol., 7 (1985) 607-614.

669.   A.S. Attiyat and G.D. Christian, Discrete Microsample Injection into a Gaseous Carrier, Talanta, 31 (1984) 463-465.

670.   W.W. De Alwis and G.S. Wilson, Rapid Sub-Picomole Electrochemical Enzyme Immunoassay for Immunoglobulin G, Anal. Chem., 57 (1985) 2754-2756.

671.   A.T. Haj-Hussein, G.D. Christian and J. Ruzicka, Determination of Cyanide by Atomic Absorption Using a Flow Injection Conversion Method, Anal. Chem., 58 (1986) 38-42.

672.   C.A. Koerner and T.A. Nieman, Chemiluminescence Flow Injection Analysis Determination of Sucrose Using Enzymatic Conversion and a Microporous Membrane Flow Cell, Anal. Chem., 58 (1986) 116-119.

673.   P. Linares, M.D. Luque de Castro and M. Valcarcel, Flow Injection Analysis of Binary and Ternary Mixtures of Arsenite, Arsenate, and Phosphate, Anal. Chem., 58 (1986) 120-124.

674.   K.W. Pratt and W.F. Koch, Determination of Trace-Level Chromium(VI) in the Presence of Chromium(III) and Iron(III) by Flow Injection Amperometry, Anal.Chem., 58 (1986) 124-127.

675.   K.R. Wehmeyer, H.B. Halsall, W.R. Heinean, C.P. Volle and I.-W. Chen, Competitive Heterogenous Enzyme Immunoassay for Digoxin with Electrochemical Detection, Anal. Chem., 58 (1986) 135-139.

676.   F. Winquist, I. Lundstrom and B. Danielsson, Determination of Creatinine by an Ammonia-Sensitive Semiconductor Structure and Immobilized Enzymes, Anal. Chem., 58 (1986) 145-148.

677.   K. Oshita, H. Wada and G. Nakagawa, Synthesis of Bidentate Pyridylazo and Thiazolylazo Reagents and the Spectrophotometric Determination of Copper in a Flow-Injection System, Anal. Chim. Acta, 176 (1985) 41-50.

678.   E.N. Chaney and R.P. Baldwin, Voltammetric Determination of Doxorubicin in Urine by Adsorptive Preconcentration and Flow Injection Analysis, Anal. Chim. Acta, 176 (1985) 105-112.

679.   P. Linares, M.D. Luque de Castro and M. Valcarcel, Spectrofluorimetric Determination of Silicon by Flow Injection Analysis, Anal.Chim. Acta, 177 (1985) 263-266.

680.   D.J. Curran and W.G. Collier, Determination of Phenyl Isocyanate in a Flow-Injection System with Infrared Spectrometric Detection, Anal. Chim. Acta, 177 (1985) 259-262.

681.   T.J. Cardwell, R.W. Cattrall and P.J. Iles, Photo-Cured Polymers in Ion-Selective Electrode Membranes. Part 2: A Calcium Electrode for Flow Injection Analysis, Anal. Chim. Acta, 177 (1985) 239-242.

682.   P.W. Alexander and P.R. Haddad, Response Characteristics of a Potentiometric Detector with a Copper Metal Electrode for Flow-Injection and Chromatographic Determinations of Metal Ions, Anal. Chim. Acta, 177 (1985) 183-195.

683.   F. Belal and J.L. Anderson, Flow Injection Analysis of Three N-Substituted Phenothiazine Drugs with Amperometric Detection at a Carbon Fibre Array Electrode, Analyst, 110 (1985) 1493-1496.

684.   S. Maspoch, M. Blanco and V. Cerda, Catalytic Determination of Manganese at Ultra-Trace Levels by Flow Injection Analysis, Analyst, 111 (1986) 69-72.

685.   A.T. Haj-Hussein and G.D. Christian, Simultaneous Spectrophotometric Determination of Copper, Nickel and Palladium by Flow Injection Analysis, Analyst, 111 (1986) 65-68.

686. M.D. Luque de Castro, Speciation Studies by Flow-Injection Analysis, Talanta, 33 (1986) 45-50.

687. J.M.H. Appleton and J.F. Tyson, Flow Injection Atomic Absorption Spectrometry: The Kinetics of Instrument Response, J. Anal. At. Spectrom., 1 (1986) 63-74.

688. J.M. Harnly and G.R. Beecher, Signal to Noise Ratios for Flow Injection Atomic Absorption Spectrometry, J. Anal. At. Spectrom., 1 (1986) 75-78.

689. J.L. Burguera, M. Burguera and O.M. Alarcon, Determination of Sodium, Potassium, Calcium, Magnesium, Iron, Copper and Zinc in Cerebrospinal Fluid by Flow Injection Atomic Absorption Spectrometry, J. Anal. At. Spectrom., 1 (1986) 79-83.

690. M. Gallego, M. Silva and M. Valcarcel, Determination of Nitrate and Nitrite by Continuous Liquid-Liquid Extraction with a Flow-Injection Atomic-Absorption Detection System, Fresenius' Z. Anal. Chem., 323 (1986) 50-53.

691. N. Ishibashi and T. Imato, Flow Injection Analysis by Using Acid-Base or Metal-Ligand Buffers and Color-Indicators, Fresenius' Z. Anal. Chem., 323 (1986) 244-248.

692. A.F. Kapauan and M.C. Magno, Continuous-Flow Injector for Flow Injection Analysis, Anal. Chem., 58 (1986) 509-510.

693. G.E. Pacey, M.R. Straka and J.R. Gord, Dual Phase Gas Diffusion Flow Injection Analysis/Hydride Generation Atomic Absorption Spectrometry, Anal. Chem., 58 (1986) 502-504.

694. R.S. Vithanage and P.K. Dasgupta, Quantitative Study of Chemical Equilibria by Flow Injection Analysis with Diode Array Detection, Anal. Chem., 58 (1986) 326-330.

695. P.I. Anagnostopoulou and M.A. Koupparis, Automated Flow-Injection Phenol Red Method for Determination of Bromide and Bromide Salts in Drugs, Anal. Chem., 58 (1986) 322-326.

696. E.H. Hansen, Flow Injection Analysis, Dissertation, Technical University of Denmark, 1986, ISBN 87-502-0636-2.

697. J.F. Coetzee and C.W. Gardner, Determination of Sulphate, Ortho-Phosphate, and Triphosphate Ions by Flow Injection Analysis with the Lead Ion Selective Electrode as Detector, Anal. Chem., 58 (1986) 608-611.

698. J.F. Coetzee and C. Gunaratna, Potentiometric Gas Sensor for the Determination of Free Chlorine in Static or Flow Injection Analysis Systems, Anal. Chem., 58 (1986) 650-653.

699. A. Rios, M.D. Luque de Castro and M. Valcarcel, New Configuration for Construction of pH Gradients in Flow Injection Analysis, Anal. Chem., 58 (1986) 663-664.

700. M.D. Luque de Castro and M. Valcarcel, Flow Injection Methods Based on Multidetection, Trends in Anal. Chem., 5 (1986) 71-74.

701. B. Olsson, H. Lundback and G. Johansson, Theory and Application of Diffusion-Limited Amperometric Enzyme Electrode Detection in Flow Injection Analysis of Glucose, Anal. Chem., 58 (1986) 1046-1052.

702. P. Van Zoonen, D.A. Kamminga, C. Gooijer, N.H. Velthorst, R.W. Frei and G. Guebitz, Quenched Peroxyoxalate Chemiluminescence as a New Detection Principle in Flow Injection Analysis and Liquid Chromatography, Anal. Chem., 58 (1986) 1245-1248.

703. A. Trojanek and S. Bruckenstein, Novel Flow-through Pneumato-Amperometric Detector for Determination of Nanogram and Subnanogram Amounts of Nitrite by Flow-Injection Analysis, Anal. Chem., 58 (1986) 866-869.

704. A.G. Fogg, R.M. Alonso and M.A. Fernandez-Arciniega, Oxidative Amperometric Flow Injection Determination of Oxalate at an Electrochemically Pre-Treated Glassy Carbon Electrode, Analyst, 111 (1986) 249-251.

705. M. Burguera and J.L. Burguera, Flow Injection-Hydride Generation System for the Determination of Arsenic by Molecular Emission Cavity Analysis, Analyst, 111 (1986) 171-174.

706. F. Lazaro, A. Rios, M.D. Luque de Castro and M. Valcarcel, Determination of Vitamin C in Urine by Flow Injection Analysis, Analyst, 111 (1986) 167-169.

707. F. Lazaro, A. Rios, M.D. Luque de Castro and M. Valcarcel, Determination of Vitamin C by Flow Injection Analysis, Analyst, 111 (1986) 163-166.

708. A.B. Ghawji and A.G. Fogg, Reduction in Size by Electrochemical Pre-Treatment at High Negative Potentials of the Background Currents Obtained at Negative Potentials at Glassy Carbon Electrodes, Analyst, 111 (1986) 157-161.

709. A. Trojanek and S. Bruckenstein, Flow-Injection Analysis of Volatile, Electroinactive Organic Compounds at a Platinum Gas Diffusion Membrane Electrode by Use of a Redox Mediator, Anal. Chem., 58 (1986) 981-982.

710. A. Trojanek and S. Bruckenstein, Flow-Injection Analysis of Phenols Via Bromination and Detection of Unreacted Bromine at a Platinized Gas Diffusion Membrane Electrode, Anal. Chem., 58 (1986) 983-985.

711. E.W. Kristensen, R.L. Wilson and R.M. Wightman, Dispersion in Flow Injection Analysis Measured with Microvoltammetric Electrodes, Anal. Chem., 58 (1986) 986-988.

712. A.N. Tsaousis and C.O. Huber, Flow-Injection Amperometric Determination of Chlorine at a Gold Electrode, Anal. Chim. Acta, 178 (1985) 319-323.

713. H.W. Shih and C.O. Huber, Amperometric Determination of Formate with a Nickel Electrode, Anal. Chim. Acta, 178 (1985) 313-317.

714. L. Gorton, A Carbon Electrode Sputtered with Palladium and Gold for the Amperometric Detection of Hydrogen Peroxide, Anal. Chim. Acta, 178 (1985) 247-253.

715. C.A. Scolari and S.D. Brown, Multicomponent Determinations in Flow-Injection Systems with Square-Wave Voltammetric Detection Using the Kalman Filter, Anal. Chim. Acta, 178 (1985) 239-246.

716.   O. Royset, Comparison of Four Chromogenic Reagents for the Flow-Injection Determination of Aluminum in Water, Anal. Chim. Acta, 178 (1985) 223-230.

717.   O. Royset, Determination of Phosphate Species in Nutrient Solutions and Phosphorus in Plant Material as Phosphovanadomolybdic Acid by Flow Injection Analysis, Anal. Chim. Acta, 178 (1985) 217-221.

718.   K. Kashiwabara, T. Hobo, E. Kobayashi and S. Suzuki, Flow Injection Analysis for Traces of Zinc with Immobilized Carbonic Anhydrase, Anal. Chim. Acta, 178 (1985) 209-215.

719.   K.K. Stewart, Time-Based Flow Injection Analysis, Anal. Chim. Acta, 179 (1986) 59-68.

720.   C. Riley, B.F. Rocks and R.A. Sherwood, Controlled-Dispersion Flow Analysis, Anal. Chim. Acta, 179 (1986) 69-80.

721.   J.N. Miller, G.L. Abdullahi, H.N. Sturley, V. Gossain and P.L. McCluskey, Studies of Interacting Biochemical Systems by Flow Injection Analysis, Anal. Chim. Acta, 179 (1986) 81-90.

722.   W.E. Van der Linden, Flow Injection Analysis in On-Line Process Control, Anal. Chim. Acta, 179 (1986) 91-101

723.   F.J. Krug, H. Bergamin Filho and E.A.G. Zagatto, Commutation in Flow Injection Analysis, Anal. Chim. Acta, 179 (1986) 103-118.

724.   J.T. Vanderslice, A.G. Rosenfeld and G.R. Beecher, Laminar-Flow Bolus Shapes in Flow Injection Analysis, Anal. Chim. Acta, 179 (1986) 119-129.

725.   J.F. Tyson, Peak Width and Reagent Dispersion in Flow Injection Analysis, Anal. Chim. Acta, 179 (1986) 131-148.

726.   M. Gisin, C. Thommen and K.F. Mansfield, Hydrodynamically Limited Precision of Gradient Techniques in Flow Injection Analysis, Anal. Chim. Acta, 179 (1986) 149-167.

727.   H.L. Pardue and P. Jager, Kinetic Treatment of Unsegmented Flow Systems. Part 3. Flow-Injection System with Gradient Chamber Evaluated with a Linearly Responding Detector, Anal. Chim. Acta, 179 (1986) 169-179.

728.   H. Wada, S. Hiraoka, A. Yuchi and G. Nakagawa, Sample Dispersion with Chemical Reaction in a Flow-Injection System, Anal. Chim. Acta, 179 (1986) 181-188.

729.   C.J. Patton and S.R. Crouch, Experimental Comparison of Flow-Injection Analysis and Air-Segmented Continuous Flow Analysis, Anal. Chim. Acta, 179 (1986) 189-201.

730.   B. Olsson, B. Stalbom and G. Johansson, Determination of Sucrose in the Presence of Glucose in a Flow-Injection System with Immobilized Multi-Enzyme Reactors, Anal. Chim. Acta, 179 (1986) 203-208.

731.   H. Hirano, Y. Baba, N. Yoza and S. Ohashi, Measurements of Kinetic Parameters of Inorganic Pyrophosphatase by Flow-Injection Procedures, Anal. Chim. Acta, 179 (1986) 209-216.

732. M. Masoom and P.J. Worsfold, The Kinetic Determination of Clinically Significant Enzymes in an Automated Flow-Injection System with Fluorescence Detection, Anal. Chim. Acta, 179 (1986) 217-223.

733. B.F. Rocks, R.A. Sherwood, M.M. Hosseinmardi and C. Riley, The Use of Holding Coils to Facilitate Long Incubation Times in Unsegmented Flow Analysis. Determination of Serum Prostatic Acid Phosphatase, Anal. Chim. Acta, 179 (1986) 225-231.

734. A.T. Faizullah and A. Townshend, Applications of Ion-Exchange Minicolumns in a Flow-Injection System for the Spectrophotometric Determination of Anions, Anal. Chim. Acta, 179 (1986) 233-244.

735. K.S. Johnson, C.L. Beehler and C.M. Sakamoto-Arnold, A Submersible Flow Analysis System, Anal. Chim. Acta, 179 (1986) 245-257.

736. G.E. Pacey, D.A. Hollowell, K.G. Miller, M.R. Straka and G. Gordon, Selectivity Enhancement by Flow Injection Analysis, Anal. Chim. Acta, 179 (1986) 259-267.

737. J.M.H. Appleton, J.F. Tyson and R.P. Mounce, The Rapid Determination of Chemical Oxygen Demand in Waste Waters and Effluents by Flow Injection Analysis, Anal. Chim. Acta, 179 (1986) 269-278.

738. F. Lazaro, A. Rios, M.D. Luque de Castro and M. Valcarcel, Simultaneous Multiwavelength Detection in Flow Injection Analysis, Anal. Chim. Acta, 179 (1986) 279-287.

739. M. Milla, R.M. De Castro, M. Garcia-Vargas and J.A. Munoz-Leyva, Batch and Flow-Injection Determination of Ethylenediamine in Pharmaceutical Preparations, Anal. Chim. Acta, 179 (1986) 289-297.

740. R.C. Schothorst, O.O. Schmitz and G. Den Boef, The Application of Strongly Oxidizing Agents in Flow Injection Analysis. Part 2. Manganese(III), Anal. Chim. Acta, 179 (1986) 299-305.

741. P.J. Worsfold and A. Nabi, Bioluminescent Assays with Immobilized Firefly Luciferase Based on Flow Injection Analysis, Anal. Chim. Acta, 179 (1986) 307-313.

742. Y. Sahlestrom and B. Karlberg, An Unsegmented Extraction System for Flow Injection Analysis, Anal. Chim. Acta, 179 (1986) 315-323.

743. Z. Fang, S. Xu, X. Wang and S. Zhang, Combination of Flow-Injection Techniques with Atomic Spectrometry in Agricultural and Environmental Analysis, Anal. Chim. Acta, 179 (1986) 325-340.

744. M. Silva, M. Gallego and M. Valcarcel, Sequential Atomic Absorption Spectrometric Determination of Nitrate and Nitrite in Meats by Liquid-Liquid Extraction in a Flow-Injection System, Anal. Chim. Acta, 179 (1986) 341-349.

745. M. Burguera, J.L. Burguera and D.M. Alarcon, Flow Injection and Microwave-Oven Sample Decomposition for Determination of Copper, Zinc and Iron in Whole Blood by Atomic Absorption Spectrometry, Anal. Chim. Acta, 179 (1986) 351-357.

746. K. Toth, J. Fucsko, E. Lindner, Zs. Feher and E. Pungor, Potentiometric Detection in Flow Analysis, Anal. Chim. Acta, 179 (1986) 359-370.

322

747.   G. Marko-Varga, R. Appelqvist and L. Gorton, A Glucose Sensor Based on Glucose Dehydrogenase Adsorbed on a Modified Carbon Electrode, Anal. Chim. Acta, 179 (1986) 371-379.

748.   G.G. Neuburger and D.C. Johnson, Constant-Potential Pulse Polarographic Detection in Flow-Injection Analysis Without Deaeration of Solvent or Sample, Anal. Chim. Acta, 179 (1986) 381-388.

749.   W. Frenzel and P. Bratter, Flow-Injection Potentiometric Stripping Analysis - A New Concept for Fast Trace Determinations, Anal. Chim. Acta, 179 (1986) 389-398.

750.   M. Masoom and A. Townshend, Flow-Injection Determination of Sulphite and Assay of Sulphite Oxidase, Anal. Chim. Acta, 179 (1986) 399-405.

751.   J.F. Van Staden, A Coated Tubular Solid-State Chloride-Selective Electrode in Flow-Injection Analysis, Anal. Chim. Acta, 179 (1986) 407-417.

752.   D.C. Stone and J.F. Tyson, Flow Cell and Diffusion Coefficient Effects in Flow Injection Analysis, Anal. Chim. Acta, 179 (1986) 427-432.

753.   M. Wasberg and A. Ivaska, A Computer-Controlled Voltammetric Flow-Injection System, Anal. Chim. Acta, 179 (1986) 433-438.

754.   M. Granados, S. Maspoch and M. Blanco, Determination of Sulphur Dioxide by Flow Injection Analysis with Amperometric Detection, Anal. Chim. Acta, 179 (1986) 445-451.

755.   T. Anfalt and S. Twengstrom, The Determination of Bromide in Natural Waters by Flow Injection Analysis, Anal. Chim. Acta, 179 (1986) 453-457.

756.   M.J. Whitaker, Spectrophotometric Determination of Nonionic Surfactants by Flow Injection Analysis Utilizing Ion-Pair Extraction and an Improved Phase Separator, Anal. Chim. Acta, 179 (1986) 459-462.

757.   A. Rios, M.D. Luque de Castro and M. Valcarcel, Simultaneous Determination by Iterative Spectrophotometric Detection in a Closed Flow System, Anal. Chim. Acta, 179 (1986) 463-468.

758.   K.H. Al-Sowdani and A. Townshend, Simultaneous Spectrofluorimetric Determination of Cerium(III) and Cerium(IV) by Flow Injection Analysis, Anal. Chim. Acta, 179 (1986) 469-473.

759.   O.F. Kamson, Spectrophotometric Determination of Iodate, Iodide and Acids by Flow Injection Analysis, Anal. Chim. Acta, 179 (1986) 475-479.

760.   S.R. Bysouth and J.F. Tyson, A Microcomputer-Based Peak-Width Method of Extended Calibration for Flow-Injection Atomic Absorption Spectrometry, Anal. Chim. Acta, 179 (1986) 481-486.

761.   A.G. Cox and C.W. McLeod, Preconcentration and Determination of Trace Chromium(III) by Flow Injection/Inductively-Coupled Plasma/Atomic Emission Spectrometry, Anal. Chim. Acta, 179 (1986) 487-490.

762.   C.G. Taylor and J.M. Trevaskis, Determination of Lead In Gasoline by a Flow-Injection Technique with Atomic Absorption Spectrometric Detection, Anal. Chim. Acta, 179 (1986) 491-496.

763.   J.L. Burguera and M. Burguera, Determination of Some Organophosphorus Insecticides by Flow Injection with a Molecular Emission Cavity Detector, Anal. Chim. Acta, 179 (1986) 497-502.

764.   J. Alonso, J. Bartroli, J.L.F.C. Lima and A.A.S.C. Machado, Sequential Flow-Injection Determinations of Calcium and Magnesium in Waters, Anal. Chim. Acta, 179 (1986) 503-508.

765.   L. Risinger, Preconcentration of Copper(II) on Immobilized 8-Quinolinol in a Flow-Injection System with an Ion-Selective Electrode Detector, Anal. Chim. Acta, 179 (1986) 509-514.

766.   H. Hwang and P.K. Dasgupta, Fluorometric Flow Injection Determination Aqueous Peroxides at Nanomolar Level Using Membrane Reactors, Anal. Chem., 58 (1986) 1521-1524.

767.   D.A. Hollowell, J.R. Gord, G. Gordon and G.E. Pacey, Selective Chlorine Dioxide Determination Using Gas-Diffusion Flow Injection Analysis with Chemiluminescent Detection, Anal. Chem., 58 (1986) 1524-1527.

768.   H. Gunasingham, B.T. Tay and K.P. Ang, Automated Mercury Film Electrode for Flow Injection Analysis and High-Performance Liquid Chromatography Detection, Anal. Chem., 58 (1986) 1578-1580.

769.   M.C. Gosnell, R.E. Snelling and H.A. Mottola, Construction and Performance of Plastic-Embedded Controlled-Pore Glass Open Tubular Reactors for Use in Continuous-Flow Systems, Anal. Chem., 58 (1986) 1585-1587.

770.   M. Koupparis, P. Macheras and C. Tsaprounis, Automated Flow-Injection Colorimetric Determination of Acetaminophen for Assay and Dissolution Studies of Multicomponent Dosage Forms, Int. J. Pharm., 27 (1985) 349-359.

771.   G. Audunsson, Aqueous/Aqueous Extraction by Means of a Liquid Membrane for Sample Cleanup and Preconcentration of Amines in a Flow System, Anal. Chem., 58 (1986) 2714-2723.

772.   R.Y. Xie and G.D. Christian, Serum Lithium Analysis by Coated Wire Lithium Ion Selective Electrodes in a Flow Injection Analysis Dialysis System, Anal. Chem., 58 (1986) 806-1810.

773.   J.D.R. Thomas, Continuous Analysis with Ion-Selective Electrodes: Flow-Injection Analysis and Monitoring of Enzyme-Based Reactions, Anal. Chem. Symp. Ser., 22 (1985) 213-229.

774.   P.K. Dasgupta and E.L. Loree, Versatile Instrument for Pulse Width Measurement, Anal. Chem., 58 (1986) 507-509.

775.   M. Yamada, S. Kamiyama and S. Suzuki, Eosin Y-Sensitized Chemiluminescence of 7,7,8,8-Tetracyanoquinodimethane in Surfactant Vesicles for Determination of Manganese(II) at Sub-Nanogram Levels by Flow Injection Method, Chem. Lett., 10 (1985) 1597-1600.

776.   M. Maeda and A. Tsuji, Chemiluminescence Flow Injection Analysis of Biological Compounds Based on the Reaction with Lucigenin, Anal. Sci., 2 (1986) 183-186.

777.   D.K. Morgan, N.D. Danielson and J.E. Katon, Aqueous Flow Injection Analysis with Fourier Transform Infrared Detection, Anal. Lett., 18 (1985) 1979-1998.

324

778.   P.J. Worsfold, Selective Biochemical Reagents in Flow Injection Analysis, Proc. Anal. Div. Chem. Soc., 22 (1985) 357-358.

779.   P. Linares, M.D. Luque de Castro and M. Valcarcel, Flow Injection Analysis in Clinical Chemistry, Rev. Anal. Chem., 8 (1985) 229-257.

780.   R. Oltner, S. Bengtsson and K. Larsson, Flow Injection Analysis for the Determination of Urea in Cow Milk, Acta Vet. Scand., 26 (1985) 396-404.

781.   I.S. Krull, Recent Advances in New and Potentially Novel Detectors in High-Performance Liquid Chromatography and Flow Injection Analysis, ACS Symp. Ser., 297 (1986) 137-166.

782.   T. Imato and N. Ishibashi, Flow Injection Analysis of Concentrated Aqueous Solution of Strong Acids and Bases, Anal. Sci., 1 (1985) 481-482.

783.   Y. Hayashi, K. Zaitsu and Y. Ohkura, Flow Injection Analysis for Hydrogen Peroxide by Using Immobilized Horseradish Peroxidase and Its Fluorogenic Substrate 3-(p-Hydroxy-phenyl)propionic Acid, Anal. Sci., 1 (1985) 65-68.

784.   K.E. LaFreniere, G.W. Rice and V.A. Fassel, Flow Injection Analysis with Inductively Coupled Plasma-Atomic Emission Spectroscopy: Critical Comparison of Conventional Pneumatic, Ultrasonic and Direct Injection Nebulization, Spectrochim. Acta, Part B, 40B (1985) 1495-1504.

785.   G. Andersson, L. Andersson and G.M. Carlstrom, Determination of Milk Urea by Flow Injection Analysis, Zentralbl. Veterinaermed., Reihe A, 33 (1986) 53-58.

786.   P.K. Dasgupta and V.K. Gupta, Membrane-Based Flow Injection System for Determination of Sulfur(IV) in Atmospheric Water, Environ. Sci. Technol., 20 (1986) 524-526.

787.   R. Klinkner and H. Engelhardt, Impact of Reaction Detectors on Flow Injection Analysis, Proc. Int. Symp. Capillary Chromatogr., 6 (1985) 857-860.

788.   J.J. Sullivan, T.A. Hollingworth, M.M. Wekell, R.T. Newton and J.E. Larose, Determination of Sulfite in Food by Flow Injection Analysis, J. Assoc. Off. Anal. Chem., 69 (1986) 542-546.

789.   F. Belal and J.L. Anderson, Flow Injection Analysis of Warfarin Sodium with Amperometric Detection, Mikrochim. Acta, 2 (1985) 145-151.

790.   A. Townshend, Recent Advances in Chemiluminescence and Flow Injection Analysis, Proc. Anal. Div. Chem. Soc., 22 (1985) 370-371.

791.   M.J. Whitaker, Industrial Applications of Flow Injection Analysis, Am. Lab., 18 (1986) 154-157.

792.   S. Alegret, J. Alonso, J. Bartroli, J.L.F.C. Lima, A.A.S.C. Machado and J.M. Paulis, Flow-through Sandwich PVC Matrix Membrane Electrode for Flow Injection Analysis, Anal. Lett., 18 (1985) 2291-2303.

793.   J. Olafsson, The Semi-Automated Determination of Manganese in Sea Water with Leuco-malachite Green, Sci. Total Environ., 49 (1986) 101-113.

794.   F. Belal and J.L. Anderson, Flow Injection Determination of Ergonovine Maleate with Amperometric Detection at the Kel-F-Graphite Composite Electrode, Talanta, 33 (1986) 448-450.

795.   A.B. Ghawji and A.G. Fogg, Reduction in Size by Electrochemical Pretreatment at High Negative Potentials of the Background Currents Obtained at Negative Potentials at Glassy Carbon Electrodes and Its Application in the Reductive Flow Injection Amperometric Determination of Nitrofurantoin, Analyst, 111 (1986) 157-161.

796.   F.T.M. Dohmen and P.C. Thijssen, A FORTH Package for Computer-Controlled Flow-Injection Analysis, Talanta, 33 (1986) 107-110.

797.   I.G. Cook, C.W. McLeod and P.J. Worsfold, Use of Activated Alumina as a Column Packing Material for Adsorption of Oxyanions in Flow Injection Analysis with ICP-AES (Inductively Coupled Plasma - Atomic Emission Spectrometry) Detection, Proc. Anal. Div. Chem. Soc., 23 (1986) 5-6.

798.   K. Matsumoto, K. Ishida and Y. Osajima, Approach for Conductometric Flow Injection Analysis of the Salt Content in Food, Nippon Shokuhin Kogyo Gakkaishi, 33 (1986) 61-66.

799.   J. Martinez Calatayud, Flow Injection Analysis of Pharmaceuticals, Pharmazie, 41 (1986) 92-95.

800.   T. Deguchi, A. Higashi and I. Sanemasa, Flow Injection Analysis of Cobalt(II) by Catalytic Oxidations of Stilbazo and Pyrocatechol Violet, Bull. Chem. Soc. Jpn., 59 (1986) 295-300.

801.   M.A. Koupparis and A. Barcuchova, Automated Flow Injection Spectrophotometric Determination of Some Phenothiazines Using Iron Perchlorate: Applications in Drug Assays, Content Uniformity, and Dissolution Studies, Analyst, 111 (1986) 313-318.

802.   F.P. Bigley, R.L. Grob and G.S. Brenner, Pharmaceutical Applications of a High-Performance Flow Injection System, Anal. Chim. Acta, 181 (1986) 241-244.

803.   J. Ruzicka and E.H. Hansen, The First Decade of Flow Injection Analysis: from Serial Assay to Diagnostic Tool, Anal. Chim. Acta, 179 (1986) 1-58.

804.   J.M. Hwang, J.C. Tsung and Y.M. Chen, Catalytic Spectrophotometric Determination of Vanadium by Flow Injection Analysis, J. Chin. Chem. Soc., 32 (1985) 405-410.

805.   J.M. Hwang and Y.M. Chen, Amperometric Determination of Chromium(III) and Chromium(IV) by Flow Injection Analysis, J. Chin. Chem. Soc., 32 (1985) 411-416.

806.   J.S. Rhee and P.K. Dasgupta, Determination of Acids, Bases, Metal Ions and Redox Species by Peak Width Measurement Flow Injection Analysis with Potentiometric, Conductometric, Fluorometric and Spectrophotometric Detection, Mikrochim. Acta, 3 (1986) 107-122.

807.   P.W. Alexander, P.R. Haddad and M. Trojanowicz, Potentiometric Flow-Injection Determination of Sugars Using a Metallic Copper Electrode, Anal. Lett., 18 (1985) 1953-1978.

808.   P. Macheras, M. Koupparis and C. Tsaprounis, An Automated Flow Injection-Serial Dynamic Dialysis Technique for Drug-Protein Binding Studies, Int. J. Pharm., 30 (1986) 123-132.

809. R. Appelqvist, G.R. Beecher, H. Bergamin Filho, G. Den Boef, J. Emneus, Z. Fang, L. Gorton, E.H. Hansen, P.E. Hare et al., Anal. Chim. Acta, 180 (1986) 1-67.

810. D.C. Johnson, S.G. Weber, A.M. Bond, R.M. Wightman, R.E. Shoup and I.S. Krull, Electroanalytical Voltammetry in Flowing Solutions, Anal. Chim. Acta, 180 (1986) 187-250.

811. J.S. Rhee and P.K. Dasgupta, Studies on Peak Width Measurement-Based FIA Acid-Base Determinations, Mikrochim. Acta., 3 (1986) 49-64.

812. H. Ishii, M. Aoki, T. Aita and T. Odashima, Flow Injection-Spectrophotometric Determination of Trace Amounts of Iron with 2-Pyridyl-3'-sulfophenylmethanone 2-Pyrimidylhydrazone and Possibility of Sensitization by Analog-Derivative Spectrophotometric Monitoring, Anal. Sci., 2 (1986) 125-129.

813. J. Ruz, A. Rios, M.D. Luque de Castro and M. Valcarcel, Flow-Injection Analysis with Multidetection as a Useful Technique for Metal Speciation, Talanta, 33 (1986) 199-202.

814. V.P.Y. Gadzekpo, G.J. Moody and J.D.R. Thomas, Lithium Ion-Selective Electrodes in Flow-Injection Analysis, Proc. Anal. Div. Chem. Soc., 23 (1986) 62-64.

815. M.D. Luque de Castro, Flow Injection Analysis: a New Tool to Automate Extraction Processes, J. Autom. Chem., 8 (1986) 56-62.

816. J.R. Chipperfield and P.J. Worsfold, A Simple Injection Valve for Flow Injection Analysis, Anal. Chim. Acta, 181 (1986) 283-285.

817. J. Ruz, A. Torres, A. Rios, M.D. Luque de Castro and M. Valcarcel, Automation of a Flow-Injection System for Multispeciation, J. Autom. Chem., 8 (1986) 70-74.

818. E.A.G. Zagatto, O. Bahia Filho, M.F. Gine and H. Bergamin Filho, A Simple Procedure for Hydrodynamic Injection in Flow Injection Analysis Applied to the Atomic Absorption Spectrometry of Chromium in Steels, Anal. Chim. Acta, 181 (1986) 265-270.

819. R.C. Schothorst and G. Den Boef, The Application of Strongly Oxidizing Agents in Flow Injection Analysis. Part 3. Cobalt(III), Anal. Chim. Acta, 181 (1986) 235-239.

820. B.A. Petersson, E.H. Hansen and J. Ruzicka, Enzymic Assay by Flow Injection Analysis with Detection by Chemiluminescence: Determination of Glucose, Creatinine, Free Cholesterol and Lactic Acid Using an Integrated FIA Microconduit, Anal. Lett., 19 (1986) 649-665.

821. A. Rios, M.D. Luque de Castro and M. Varcalcel, Determination of Reaction Stoichiometries by Flow-Injection Analysis. A Laboratory Exercise, J. Chem. Educ., 63 (1986) 552-553.

822. M. Gallego, M. Silva and M. Valcarcel, Indirect Atomic Absorption Determination of Anionic Surfactants in Wastewaters by Flow Injection Continuous Liquid-Liquid Extraction, Anal. Chem., 58 (1986) 2265-2269.

823. D. Betteridge, T.J. Sly, A.P. Wade and D.G. Porter, Versatile Automatic Development System for Flow Injection Analysis, Anal. Chem., 58 (1986) 2258-2265.

824. S.D. Hartenstein, G.D. Christian and J. Ruzicka, Applications of an On-Line Preconcentrating Flow Injection Analysis System for Inductively Coupled Plasma Atomic Emission Spectrometry, Can. J. Spectrosc., 30 (1985) 144-148.

825. J. Toei and N. Baba, Multifunction Valve for Flow Injection Analysis, Anal. Chem., 58 (1986) 2348-2350.

826. T. Komatsu, M. Ohira, M. Yamada and S. Suzuki, Analytical Use of Luminescence Induced Ultrasonically in Solution. I. Sonic Chemiluminescence of Luminol for Determination of Cobalt(II) at Sub-pg Levels by Flow Injection and Continuous Flow Methods, Bull. Chem. Soc. Jpn., 59 (1986) 1849-1855.

827. M. Burguera, J.L. Burguera, P.C. Rivas and O.M. Alarcon, Determination of Copper, Zinc, and Iron in Parotid Saliva by Flow Injection with Flame Atomic Absorption Spectrophotometry, At. Spectrosc., 7 (1986) 79-81.

828. S.V. Olesik, S.B. French and M. Novotny, Reaction Monitoring in Supercritical Fluids by Flow Injection Analysis with Fourier Transform Infrared Spectrometric Detection, Anal. Chem., 58 (1986) 2256-2258.

829. T.D. Yerian, T.P. Hadjiioannou, G.D. Christian, Flow-Injection Analysis with the Iron-Induced Perbromate-Iodide Reaction: Spectrophotometric Determination of Iron, Talanta, 33 (1986) 547-549.

830. A.T. Haj-Hussein and G.D. Christian, Multicomponent Flow Injection Analysis Using Spectrophotometric Detection with Reagent Spectral Overlap: Application to Determination of Calcium and Magnesium in Blood Serum Using Eriochrome Black T, Microchem. J., 34 (1986) 67-75.

831. M.H. Memon and P.J. Worsfold, The Use of Microemulsions in Flow Injection Analysis. Spectrofluorometric Determination of Primary Amines, Anal. Chim. Acta, 183 (1986) 179-185.

832. V.P.Y. Gadzekpo, G.J. Moody and J.D.R. Thomas, Problems in the Application of Ion-Selective Electrodes to Serum Lithium Analysis, Analyst, 111 (1986) 567-570.

833. N. Leon, J.L. Burguera, M. Burguera and O.M. Alarcon, Determination of Cobalt and Manganese in Blood Serum by Flow Injection Analysis and Atomic Absorption Spectroscopy, Rev. Roum. Chim., 31 (1986) 353-360.

834. C.M. Sakamoto-Arnold, K.S. Johnson and C.L. Beehler, Determination of Hydrogen Sulfide in Seawater Using Flow Injection Analysis and Flow Analysis, Limnol. Oceanogr., 31 (1986) 894-900.

835. B.A. Woods, J. Ruzicka, G.D. Christian and R.J. Charlson, Measurement of pH in Solutions of Low Buffering Capacity and Ionic Strength by Optosensing Flow Injection Analysis, Anal. Chem., 58 (1986) 2496-2502.

836. J.A. Koropchak and D.H. Winn, Thermospray Interfacing for Flow Injection Analysis with Inductively Coupled Plasma Atomic Emission Spectrometry, Anal. Chem., 58 (1986) 2558-2561.

328

837. M. Bengtsson, F. Malamas, A. Torstensson, O. Regnell and G. Johansson, Trace Metal Ion Preconcentration for Flame Atomic Absorption by an Immobilized N,N,N'-Tri-(2-pyridylmethyl) Ethylenediamine (TriPEN) Chelate Ion Exchanger in a Flow Injection System, Mikrochim. Acta, 3 (1986) 209-221.

838. J.J. Thompson and R.S. Houk, Inductively Coupled Plasma Mass Spectrometric Detection for Multielement Flow Injection Analysis and Elemental Speciation by Reversed-Phase Liquid Chromatography, Anal. Chem., 58 (1986) 2541-2548.

839. T. Fukasawa, S. Kawakubo and A. Unno, Flow-Injection Spectrophotometric Determination of Trace Vanadium Based on Catalysis of the Gallic Acid Bromate Reaction, Anal. Chim. Acta, 183 (1986) 269-274.

840. C.E. Lomen, U. De Alwis and G.S. Wilson, Use of a Reversibly Immobilized Enzyme in the Flow Injection-Amperometric Determination of Picomole Glucose Levels, J. Chem. Soc. Faraday Trans. 1, 82 (1986) 1265-1270.

841. J. Ruzicka, Flow Injection Analysis - A Survey of Its Potential for Spectroscopy, Fresenius' Z. Anal. Chem., 324 (1986) 745-749.

842. J.T. Dobbins and J.M. Martin, Flow Injection Analysis: Twelve Years Old and Growing, Spectroscopy, 1 (1986) 20-29.

843. A.G. Fogg, Reverse Flow Injection Formation of Monitorands in Direct and Indirect Online Flow Injection Analysis Methods, Analyst, 111 (1986) 859-860.

844. C. Pasquini and W.A. De Oliveira, Determination of Iron in Iron Ores Using Enthalpimetric Flow Injection Analysis, Analyst, 111 (1986) 856-858.

845. G.J. Moody, G.S. Sanghera and J.D.R. Thomas, Amperometric Enzyme Electrode System for the Flow Injection Analysis of Glucose, Analyst, 111 (1986) 605-609.

846. R.W. Abbott, A. Townshend and R. Gill, Determination of Morphine by Flow Injection Analysis with Chemiluminescence Detection, Analyst, 111 (1986) 635-649.

847. H. Wada, Y. Deguchi and G. Nakagawa, Determination of Calcium in Chlor-Alkali Brine by Flow Injection Analysis, Mikrochim. Acta, 3 (1986) 395-402.

848. J. Hernandez-Mendez, A. Alonso Mateos, M.J. Almendral Parra and C. Garcia de Maria, Spectrophotometric Flow-Injection Determination of Ascorbic Acid by Generation of Triiodide, Anal. Chim. Acta, 184 (1986) 243-250.

849. J.A. Osborn, A.M. Yacynych and D.C. Roberts, A Flow-Injection System for Assay of the Activity of an Immobilized Enzyme Chemically-Modified Electrode, Anal. Chim. Acta, 183 (1986) 287-292.

850. E.B. Milosavljevic, L. Solujic, J.H. Nelson and J.L. Hendrix, Simultaneous Determination of Chromium(VI) and Chromium(III) by Flame Atomic Absorption Spectrometry with the Chelating Ion-Exchange Flow Injection System, Mikrochim. Acta, 3 (1986) 353-360.

851. J.F. Tyson, J.R. Mariara and J.M.H. Appleton, A Variable Dispersion Flow Injection Manifold for Calibration and Sample Dilution in Flame Atomic Absorption Spectrometry, J. Anal. At. Spectrom., 1 (1986) 273-276.

852.   M. Aihara, M. Arai and T. Taketatsu, Flow Injection Spectrofluorometric Determination of Europium(III) Based on Solubilizing Its Ternary Complex with Thenoyltrifluoro-Acetone and Trioctylphosphine Oxide in Micellar Solution, Analyst, 111 (1986) 641-643.

853.   B.A. Petersson, Z. Fang, J. Ruzicka and E.H. Hansen, Conversion Techniques in Flow Injection Analysis. Determination of Sulfide by Precipitation with Cadmium Ions and Detection by Atomic Absorption Spectrometry, Anal. Chim. Acta, 184 (1986) 165-172.

854.   E.G. Sarantonis and A. Townshend, Flow-Injection Determination of Iron(II), Iron(III), and Total Iron with Chemiluminescence Detection, Anal. Chim. Acta, 184 (1986) 311-315.

855.   F. Lazaro, M.D. Luque de Castro and M. Valcarcel, Photometric Determination of Tartaric Acid in Wine by Flow Injection Analysis, Analyst, 111 (1986) 729-732.

856.   K. Matsumoto, O. Hamada, H. Ukeda and Y. Osajima, Amperometric Flow Injection Determination of Fructose with an Immobilized Fructose 5-Dehydrogenase Reactor, Anal. Chem., 58 (1986) 2732-2734.

857.   J.W. Keller, T.F. Gould and K.T. Aubert, The ASYST Language for the IBM PC: an Application to Flow Injection Analysis, J. Chem. Educ., 63 (1986) 709-711.

858.   M.H. Ho, Automated Flow Injection Analysis System for Formaldehyde Determination, ACS Symp. Ser., 316 (1986) 107-115.

859.   C.A. Lucy and F.F. Cantwell, Simultaneous Determination of Phenylephrine Hydrochloride and Pheniramine Maleate in Nasal Spray by Solvent Extraction-Flow Injection Analysis Using Two Porous-Membrane Phase Separators and One Photometric Detector, Anal. Chem., 58 (1986) 2727-2731.

860.   J. Martinez Calatayud and C. Falco, Determination of Levamisole Hydrochloride with Tetraiodomercurate-(2-) by a Turbidimetric Method and Flow-Injection Analysis, Talanta, 33 (1986) 685-687.

861.   S. Hirata, Y. Umezaki and M. Ikeda, Determination of Chromium(III), Titanium, Vanadium, Iron(III), and Aluminum by Inductively Coupled Plasma Atomic Emission Spectrometry with an On-Line Preconcentrating Ion-Exchange Column, Anal. Chem., 58 (1986) 2602-2606.

862.   P.K. Dasgupta and H.C. Yang, Trace Determination of Aqueous Sulfite, Sulfide and Methanethiol by Fluorometric Flow Injection Analysis, Anal. Chem., 58 (1986) 2839-2844.

863.   J.A. Cox and K.R. Kulkarni, Flow Injection Determination of Nitrite by Amperometric Detection at a Modified Electrode, Analyst, 111 (1986) 1219-1220.

864.   L. Ilcheva and K. Cammann, A Simple, Selective and Sensitive Liquid Chromatographic or Flow-Injection Detector for Chloroorganic Compounds Based on Ion-Selective Electrodes, Fresenius' Z. Anal. Chem., 325 (1986) 11-14.

330

865.   J.W. Bixler and A.M. Bond, Amperometric Detection of Picomole Samples in a Microdisk Electrochemical Flow-Jet Cell with Dilute Supporting Electrolyte, Anal. Chem., 58 (1986) 2859-2863.

866.   P.E. Macheras and M.A. Koupparis, An Automated Flow-Injected Serial Dynamic Dialysis Technique for Drug-Protein Binding Studies, Anal. Chim. Acta, 185 (1986) 65-73.

867.   S. Motomizu, H. Mikasa and K. Toei, Fluorometric Determination of Nitrite in Natural Waters with Aminonaphthalene-1,5-disulfonic Acid by Flow-Injection Analysis, Talanta, 33 (1986) 729-732.

868.   R.Y. Xie, V.P.Y. Gadzekpo, A.M. Kadry, Y.A. Ibrahim, J. Ruzicka and G.D. Christian, Use of a Flow-Injection System in the Evaluation of the Characteristic Behavior of Neutral Carriers in Lithium Ion-Selective Electrodes, Anal. Chim. Acta, 184 (1986) 259-269.

869.   P. Martinez-Jimenez, M. Gallego and M. Valcarcel, Indirect Atomic Absorption Determination of Aluminum by Flow Injection Analysis, Microchem. J., 34 (1986) 190-195.

870.   W. Frenzel and P. Braetter, The Fluoride Ion-Selective Electrode in Flow Injection Analysis. Part 1. General Methodology, Anal. Chim. Acta, 185 (1986) 127-136.

871.   K. Wolf and P.J. Worsfold, Flow Injection Analysis as a Sample Handling Technique for Diode Array Spectroscopy, Proc. Anal. Div. Chem. Soc., 23 (1986) 365-366.

872.   J. Pawliszyn, Properties and Applications of the Concentration Gradient Sensor to Detection of Flowing Samples, Anal. Chem., 58 (1986) 3207-3215.

873.   M. Masoom and A. Townshend, Cyclic Regeneration of Nicotinamide Adenine Dinucleotide with Immobilized Enzymes. Flow-Injection Spectrofluorometric Determination of Ethanol, Anal. Chim. Acta, 185 (1986) 49-55.

874.   O. Royset, Flow-Injection Spectrophotometric Determination of Aluminum in Water with Pyrocatechol Violet, Anal. Chim. Acta, 185 (1986) 75-81.

875.   D.E. Davey, D.E. Mulcahy and G.R. O'Connell, Rapid Determination of Fluoride in Potable Waters by Potentiometric Flow Injection Analysis, Anal. Lett., 19 (1986) 1387-1406.

876.   A.A. Alwarthan and A. Townshend, Chemiluminescence Determination of Buprenorphine Hydrochloride by Flow Injection Analysis, Anal. Chim. Acta, 185 (1986) 329-333.

877.   F. Lazaro, A. Rios, M.D. Luque de Castro and M. Valcarcel, Diode Array Detectors in Hydrodynamic Analytical Systems, Analusis, 14 (1986) 378-388.

878.   J.F. Van Staden, Electrodes in Series. Simultaneous Flow Injection Determination of Chloride and pH with Ion-Selective Electrodes, Analyst, 111 (1986) 1231-1234.

879.   M.A. Koupparis and P.I. Anagnostopoulou, Automated Flow Injection Spectrophotometric Determination of Zinc Using Zincon: Application to Analysis of Waters, Alloys and Insulin Formulations, Analyst, 111 (1986) 1311-1315.

880.   D.E. Davey, The Analysis of Galvanizing Preflux Solutions for Zinc by Flow Injection-Flame Atomic Absorption Spectrometry, Anal. Lett., 19 (1986) 1573-1590.

881.   M. Ishii, M. Yamada and S. Suzuki, Didodecyldimethylammonium Bromide Bilayer Vesicle-Catalyzed and Uranine-Sensitized Chemiluminescence for Determination of Free Cyanide at Picogram Levels by Flow Injection Method, Anal. Lett., 19 (1986) 1591-1601.

882.   T.Z. Polta and D.C. Johnson, Pulsed Amperometric Detection of Sulfur Compounds. Part I. Initial Studies at Platinum Electrodes in Alkaline Solutions, J. Electroanal. Chem. Interfacial Electrochem., 209 (1986) 159-169.

883.   N.H. Tioh, Y. Israel and R.M. Barnes, Determination of Arsenic in Glycerine by Flow Injection, Hydride Generation and Inductively-Coupled Plasma/Atomic Emission Spectrometry, Anal. Chim. Acta, 184 (1986) 205-212.

884.   F. Lazaro, M.D. Luque de Castro and M. Valcarcel, Individual and Simultaneous Enzymic Determination of Ethanol and Acetaldehyde in Wines by Flow Injection Analysis, Anal. Chim. Acta, 185 (1986) 57-64.

885.   J.D. Trent and S. Christiansen, Determination of Total Nonstructural Carbohydrates in Forage Tissue by p-Hydroxybenzoic Acid Hydrazide Flow Injection Analysis, J. Agric. Food Chem., 34 (1986) 1033-1037.

886.   W.A. McCrum, The Determination of Dissolved Humic Substances in River Waters Using Flow Injection Analysis with Fluorometric Detection, Proc. Anal. Div. Chem. Soc., 23 (1986) 307.

887.   T. Iwachido, M. Onoda and S. Motomizu, Determination of Potassium in River Water by Solvent Extraction-Flow Injection Analysis, Anal. Sci., 2 (1986) 493-494.

888.   J.F. Van Staden, A.E. Joubert and H.R. Van Vliet, Flow Injection Determination of Nitrate in Natural Waters with Copper- and Copperized Cadmium Tubes in the Reaction Manifold System, Fresenius' Z. Anal. Chem., 325 (1986) 150-152.

889.   K. Stulik and V. Pacakova, Some Aspects of Design, Performance and Applications of Electrochemical Detectors in HPLC and FIA, Ann. Chim., 76 (1986) 315-332.

890.   J.F. Van Staden, Incorporation of a Coated Tubular Solid-State Iodide-Selective Electrode into the Conduits of a Flow-Injection System, Fresenius' Z. Anal. Chem., 325 (1986) 247-251.

891.   A.Y. Chamsi and A.G. Fogg, Application of the Reductive Flow Injection Amperometric Determination of Iodine at a Glassy Carbon Electrode to the Iodometric Determination of Hypochlorite and Hydrogen Peroxide, Analyst, 111 (1986) 879-882.

892.   J.F. Tyson, Extended Range Calibrations by Flow Injection Analysis, Proc. Anal. Div. Chem. Soc., 23 (1986) 304-305.

893.   G.J. Moody, G.S. Sanghera and J.D.R. Thomas, Modified Platinum Wire Glucose Oxidase Amperometric Electrode, Analyst, 111 (1986) 1235-1239.

894. F. Morishita, Y. Nishikawa and T. Kojima, Simultaneous Determination of Three Species by Flow Injection Analytical Method Using Enzyme-Immobilized Open-Tubular Reactors, Anal. Sci., 2 (1986) 411-415.

895. P. Hernandez, L. Hernandez and J. Losada, Determination of Aluminium in Hemodialysis Fluids by a Flow Injection System with Preconcentration on a Synthetic Chelate-Forming Resin and Flame Atomic Absorption Spectrophotometry, Fresenius' Z. Anal. Chem., 325 (1986) 300-302.

896. K.A. Holm, Automatic Spectrophotometric Determination of Amyloglucosidase Activity Using p-Nitrophenyl-α-D-glucopyranoside and a Flow Injection Analyzer, Analyst, 111 (1986) 927-929.

897. T.D. Yerican, G.D. Christian and J. Ruzicka, Enzymic Determination of Urea in Water and Serum by Optosensing Flow Injection Analysis, Analyst, 111 (1986) 865-873.

898. J.F. Van Staden, Flow-Injection Analysis of Chloride in Milk with a Dialyzer and a Coated Tubular Inorganic Chloride-Selective Electrode, Anal. Lett., 19 (1986) 1407-1419.

899. B. Bouzid and A.M.G. Macdonald, Flow Injection Determination of 5-Fluorouracil with Voltammetric Detection, Proc. Anal. Div. Chem. Soc., 23 (1986) 295-297.

900. Y. Sahlestrom and B. Karlberg, Flow-Injection Extraction with a Microvolume Module Based on Integrated Conduits, Anal. Chim. Acta., 185 (1986) 259-269.

901. P. Linares, M.D. Luque de Castro and M. Valcarcel, Spectrophotometric Determination of Selenium(IV) and Selenium(VI) with Flow Injection, Analyst, 111 (1986) 1405-1407.

902. A. Nabi and P.J. Worsfold, Bioluminescence Assays with Immobilized Bacterial Luciferase Using Flow Injection Analysis, Analyst, 111 (1986) 1321-1324.

903. B.C. Madsen and D.W. Doller, Continuous-Flow Real-Time and Flow Injection Determination of Rainwater pH with a Poly(vinyl Chloride)-Based Tubular Membrane Electrode, Anal. Chem., 59 (1987) 127-130.

904. P. Linares, M.D. Luque de Castro and M. Valcarcel, Fluorometric Differential-Kinetic Determination of Silicate and Phosphate in Waters by Flow-Injection Analysis, Talanta, 33 (1986) 889-893.

905. G.B. Martin and M.E. Meyerhoff, Membrane-Dialyzer Injection Loop for Enhancing the Selectivity of Anion-Responsive Liquid-Membrane Electrodes in Flow Systems. Part 1. A Sensing System for Nitrogen Oxide and Nitrite, Anal. Chim. Acta, 186 (1986) 71-80.

906. T.J. Cardwell, R.W. Cattrall, P.C. Hauser and I.C. Hamilton, Buffer Systems for Use with the Fluoride Electrode in Flow-Injection Analysis, Anal. Chem., 59 (1987) 206-208.

907. P. Martinez-Jimenez, M. Gallego and M. Valcarcel, Analytical Potential of Continuous Precipitation in Flow Injection-Atomic Absorption Configurations, Anal. Chem., 59 (1987) 69-74.

908. Nussbaum, H.L. Nekimken and T.A. Nieman, Luminol Chemiluminescence for Determination of Iron(II) in Ferrioxalate Chemical Actinometry, Anal. Chem., 59 (1987) 211-212.

909. J. Ruz, A. Rios, M.D. Luque de Castro and M. Valcarcel, Flow-Injection Configurations for Chromium Speciation with a Single Spectrophotometric Detector, Anal. Chim. Acta, 186 (1986) 139-146.

910. G.G. Neuburger and D.C. Johnson, Comparison of the Pulsed Amperometric Detection of Carbohydrates at Gold and Platinum Electrodes for Flow Injection and Liquid Chromatographic Systems, Anal. Chem., 59 (1987) 203-204.

911. G.G. Neuburger and D.C. Johnson, Pulsed Amperometric Detection of Carbohydrates at Gold Electrodes with a Two-Step Potential Waveform, Anal. Chem., 59 (1987) 150-154.

912. S.R. Mikkelsen and W.C. Purdy, Coulostatic Electrochemical Detection in Flow Injection Analysis, Anal. Chem., 59 (1987) 244-247.

913. Q. Chang and M.E. Meyerhoff, Membrane-Dialyzer Injection Loop for Enhancing the Selectivity of Anion-Responsive Liquid-Membrane Electrodes in Flow Systems. Part 2. A Selective Sensing System for Salicylate, Anal. Chim. Acta, 186 (1986) 81-90.

914. D. Taylor and A.T. Nieman, Bipolar Pulse Conductometric Detection of Enzyme Reactions in Flow-Injection Systems. Urea in Serum and Urine, Anal. Chim. Acta, 186 (1986) 91-100.

915. F. Scheller and F. Schubert, Flow Injection Analysis of Lactate and Lactate Dehydrogenase Using an Enzyme Membrane in Conjunction with a Modified Electrode, Anal. Lett., 19 (1986) 1691-1703.

916. P. Macheras, M. Koupparis and C. Tsaprounis, Drug Dissolution Studies in Milk Using the Automated Flow Injection Serial Dynamic Dialysis Technique, Int. J. Pharm., 33 (1986) 125-136.

917. B. Hoeyer and L. Kryger, Evaluation of Single-Point Calibration in Flow Potentiometric Stripping Analysis, Talanta, 33 (1986) 883-888.

918. J.L. Burguera, M. Burguera, O.L. La Cruz, and O.R. Naranjo, Determination of Lead in the Urine of Exposed and Unexposed Adults by Extraction and Flow-Injection/Atomic Absorption Spectrometry, Anal. Chim. Acta, 186 (1986) 273-277.

919. Y. Hayashi, K. Zaitsu and Y. Ohkura, Flow-Injection Determination of Adenosine and Inosine in Blood Plasma with Immobilized Enzyme Columns Connected in Series and Fluorometric Detection, Anal. Chim. Acta, 186 (1986) 131-137.

920. K.W. Simonsen, B. Nielsen, A. Jensen and J.R. Andersen, Direct Microcomputer Controlled Determination of Zinc in Human Serum by Flow Injection Atomic Absorption Spectrometry, J. Anal. At. Spectrom., 1 (1986) 453-456.

921. A. Rios, M.D. Luque de Castro, M. Valcarcel and H.A. Mottola, Electrochemical Determination of Sulfur Dioxide in Air Samples in Closed-Loop Flow Injection System, Anal. Chem., 59 (1987) 666-670.

334

922.   M. Aihara, M. Arai and T. Tomitsugu, Flow Injection Spectrofluorometric Determination of Terbium(III) Based on Solubilizing its Ternary Complex in Micellar Solution, Anal. Lett., 19 (1986) 1907-1916.

923.   D. Eadington and J.A.W. Dalziel, Reductive Determination of Metal Ions by Flow Injection Analysis Using Amperometric Amalgam Detectors, Proc. Anal. Div. Chem. Soc., 23 (1986) 434-435.

924.   G.J. Moody, G.S. Sanghera and J.D.R. Thomas, Enzyme Electrode Systems for Glucose Determination, Proc. Anal. Div. Chem. Soc., 23 (1986) 446-448.

925.   P. Van Zoonen, I. De Herder, C. Gooijer, N.H. Velthorst, R.W. Frei, E. Kuentzberg and G. Guebitz, Detection of Oxidase Generated Hydrogen Peroxide by a Solid State Peroxyoxalate Chemiluminescence Detector, Anal. Lett., 19 (1986) 1949-1961.

926.   G.J. Moody, G.S. Sanghera and J.D.R. Thomas, Chemically Immobilized Bi-Enzyme Electrodes in the Redox Mediated Mode for the Flow Injection Analysis of Glucose and Hypoxanthine, Analyst, 112 (1987) 65-70.

927.   A. Nabi and P.J. Worsfold, Indirect Assays with Immobilized Firefly Luciferase Based on Flow Injection Analysis, Proc. Anal. Div. Chem. Soc., 23 (1986) 415-416.

928.   M.D. Luque de Castro and M. Valcarcel, Kinetic-Based Determinations in Continuous-Flow Analysis, J. Autom. Chem., 8 (1986) 186-191.

929.   J. Martinez Calatayud, M.C. Pascual Marti and S. Sagrado Vives, Determination of Paracetamol by a Flow Injection-Spectrophotometric Method, Anal. Lett., 19 (1986) 2023-2038.

930.   J. Martinez Calatayud, P. Campins Falco and A. Sanchez Sampedro, Turbidimetric Determination of Chlorhexidine Using Flow Injection Analysis, Analyst, 112 (1987) 87-90.

931.   M.A. Koupparis and E.G. Sarantonis, Automated Flow-Injection Mercurothiocyanate Determination of Chloride Salts of Drugs for Routine Assays: Content Uniformity and Dissolution Studies, J. Pharm. Sci., 75 (1986) 800-804.

932.   J.A. Cox and K.R. Kulkarni, Comparison of Inorganic Films and Poly(4-vinylpyridine) Coatings as Electrode Modifiers for Flow-Injection Systems, Talanta, 33 (1986) 911-913.

933.   A. Al-Wehaid and A. Townshend, Spectrophotometric Flow-Injection Determination of Nitrate Based on Reduction with Titanium(III) Chloride, Anal. Chim. Acta, 186 (1986) 289-294.

934.   C.E. Lunte, T.H. Ridgway and W.R. Heineman, Voltammetric-Amperometric Dual-Electrode Detection for Flow Injection Analysis and Liquid Chromatography, Anal. Chem., 59 (1987) 761-766.

935.   J. Wang, T. Golden and T. Peng, Poly(4-vinylpyridine)-Coated Glassy Carbon Flow Detectors, Anal. Chem., 59 (1987) 740-744.

936.   R.Y. Xie and G.D. Christian, Lithium Ion-Selective Electrodes Containing TOPO: Determination of Serum Lithium by Flow Injection Analysis, Analyst, 112 (1987) 61-64.

937.   M. Kopanica and V. Stara, Voltammetric Determination of Cyadox Using Adsorptive Accumulation in a Flow-through System, J. Electroanal. Chem. Interfacial Electrochem., 214 (1986) 115-123.

938.   O. Royset, Flow Injection Spectrophotometric Determination of Aluminum in Natural Water Using Eriochrome Cyanine R and Cationic Surfactants, Anal. Chem., 59 (1987) 899-903.

939.   K. Hool and T.A. Nieman, Chemiluminescence Analysis in Flowing Streams with Luminol Immobilized on Silica and Controlled-Pore Glass, Anal. Chem., 59 (1987) 869-872.

940.   A. Rios, M.D. Luque de Castro and M. Valcarcel, Simultaneous Flow-Injection Fluorometric Determination of Ammonia and Hydrazine with a Novel Mode of Forming pH Gradients, Anal. Chim. Acta, 187 (1986) 139-145.

941.   J.M. Hwang, T.S. Wei and Y.M. Chen, Catalytic Photometric Determination of Selenium by Flow Injection Analysis, J. Chin. Chem. Soc., 33 (1986) 109-115.

942.   Y. Sahlestrom, S. Twengstrom and B. Karlberg, An Automated Flow-Injection Extraction Method for Determination of Bittering Compounds in Beer, Anal. Chim. Acta, 187 (1986) 339-342.

943.   B.G. Osborne, Determination of Potassium Bromate in Flour by Flow Injection Analysis, Analyst, 112 (1987) 137-139.

944.   N. Yoza, Y. Sagara, H. Morioka, T. Handa, H. Hirano, Y. Baba and S. Ohashi, Fate Analysis of Phosphorus Compounds in Environmental Waters by Flow Injection Analysis and High-Performance Liquid Chromatography, J. Flow Injection Anal., 3 (1986) 37-46.

945.   R.M. Alonso, R.M. Jimenez, M.A. Fernandez-Arciniega and L. Hernandez, Flow Injection Amperometric Determination of Clotiazepam at a Glassy Carbon Electrode, Anal. Chem. Symp. Ser., 25 (1986) 397-401.

946.   F. Canate, A. Rios, M.D. Luque de Castro and M. Valcarcel, Determination of Analytical Parameters in Drinking Water by Flow Injection Analysis. Part 1. Simultaneous Determination of pH, Alkalinity, and Total Ionic Concentration, Analyst, 112 (1987) 263-266.

947.   F. Canete, A. Rios, M.D. Luque de Castro and M. Valcarcel, Determination of Analytical Parameters in Drinking Water by Flow Injection Analysis. Part 2. Simultaneous Determination of Calcium and Magnesium, Analyst, 112 (1987) 267-270.

948.   J. Ruzicka, Flow Injection Analysis Revisited, Chemtech, 17 (1987) 96-97.

949.   P. Jager and H.L. Pardue, Kinetic Treatment of Unsegmented Flow Systems. Part 4. Equations for a System with Gradient Chamber Corrected to Account for Detectors with Finite Sensitivities, Anal. Chim. Acta, 187 (1986) 343-346.

950.   B.C. Erickson, B.R. Kowalski and J. Ruzicka, Applications of a Versatile Injection Valve for Flow Injection Analysis, Anal. Chem., 59 (1987) 1246-1248.

951.   W. Frenzel, G. Welter and P. Braetter, Flow Injection Analysis for Electrochemical Stripping. Comparison of Different Techniques, Anal. Chem. Symp. Ser., 25 (1986) 77-82.

952.   A. Nabi and P.J. Worsfold, A Kinetic Method for the Spectrophotometric Determination of Cobalt Using Flow Injection Analysis, J. Chem. Soc. Pak., 8 (1986) 487-490.

953.   G.E. Pacey, Y.P. Wu and K. Sasaki, Selective Determination of Lithium in Biological Fluids Using Flow Injection Analysis, Anal. Biochem., 160 (1987) 243-250.

954.   C.E. Lunte, W.R. Heineman, H.B. Halsall, K.R. Wehmeyer, M.J. Doyle and D.S. Wright, Immunoassay by HPLC and Flow Injection Analysis with Electrochemical Detection, Proc. Electrochem. Soc., 86-14 (1986) 129-138.

955.   L.C. Davis and G.A. Radke, Measurement of Protein Using Flow Injection Analysis with Bicinchoninic Acid, Anal. Biochem., 161 (1987) 152-156.

956.   K.A. Holm, Spectrophotometric Flow-Injection Determination of Cellobiose Dehydrogenase Activity in Fermentation Samples with 2,6-Dichlorophenolindophenol, Anal. Chim. Acta, 188 (1986) 285-288.

957.   Y.W. Feng and C.O. Huber, Solvent Extraction Using a Polymer as Solvent with an Amperometric Flow-Injection Detector, Adv. Chem. Ser., 214 (1987) 349-357.

958.   R.A. Kenley, S.E. Jackson, G.C. Visor and J.S. Winterle, Automated Dissolution Testing with Flow-Injection Analysis. Dissolution Profiles for the Antiviral Drugs, DHPG and Acyclovir, in Capsule Formulations, Drug Dev. Ind. Pharm., 13 (1987) 39-56.

959.   H. Hwang and P.K. Dasgupta, Tubular Microporous Membrane Entrapped Enzyme Reactors for Flow Injection Analysis, Anal. Chem., 59 (1987) 1356-1360.

960.   D. Betteridge, P.B. Oates and A.P. Wade, Control of Dispersion and Variation of Reaction Coil Length in Flow Injection Analyzers by Flow Reversals, Anal. Chem., 59 (1987) 1236-1238.

961.   C.E. Lunte, S.W. Wong, T.H. Ridgway, W.R. Heineman and K.W. Chan, Voltammetric/Amperometric Detection for Flow-Injection Systems, Anal. Chim. Acta, 188 (1986) 263-267.

962.   W. Frenzel and P. Braetter, The Fluoride Ion-Selective Electrode in Flow Injection Analysis. Part 3. Applications, Anal. Chim. Acta, 188 (1986) 151-164.

963.   M. Trojanowicz, A. Hulanicki, W. Matuszewski, M. Palys, A. Fuksiewicz, T. Hulanicka-Michalak, S. Raszewski, J. Szyller and W. Augustyniak, Flow-Injection Catalytic Determination of Molybdenum with Biamperometric Detection in a Microprocessor-Controlled System, Anal. Chim. Acta, 188 (1986) 165-175.

964.   J. Ruz, M.D. Luque de Castro and M. Valcarcel, Flow Injection Analysis-Use of Immobilized Enzymes for the Determination of Ethanol in Serum, Analyst, 112 (1987) 259-261.

965.   M. Masoom and P.J. Worsfold, Automated Flow-Injection Procedures for Glycerol and Triglycerides, Anal. Chim. Acta, 188 (1986) 281-283.

966.   J.F. Tyson, Analytical Information from Doublet Peaks in Flow Injection Analysis. Part II. Determination of Stability Constants, Analyst, 112 (1987) 527-529.

967.   K. Backstrom, L.-G. Danielsson and L. Nord, Dispersion in Phase Separators for Flow-Injection Extraction Systems, Anal. Chim. Acta, 187 (1986) 255-269.

968.   T. Kakizaki, K. Hasebe and H. Yoshida, Evaluation of Dispersion in a Flow Injection System by Electrochemical Detector and Personal Computer, Fresenius' Z. Anal. Chem., 326 (1987) 214-217.

969.   W. Frenzel and P. Braetter, Fluoride Ion-Selective Electrode in Flow-Injection Analysis. Part 2. Interference Studies, Anal. Chim. Acta, 187 (1986) 1-16.

970.   B. Bermudez, F. Lazaro, M.D. Luque de Castro and M. Valcarcel, Simultaneous Determination of Organic Isomers in Mixtures by Flow Injection Analysis with a Diode Array Photodetector, Analyst, 112 (1987) 535-538.

971.   A. Nabi and P.J. Worsfold, Flow Injection Procedures for the Determination of Ethanol and Alcohol Dehydrogenase Using Coimmobilized Bacterial Luciferase and Oxidoreductase, Analyst, 112 (1987) 531-533.

972.   M.M. Cooper and S.R. Spurlin, Chemiluminescent Method for Flow Injection Analysis of Anions, Anal. Lett., 19 (1986) 2221-2230.

973.   M.P. Granchi, J.A. Biggerstaff, L.J. Hilliard and P. Grey, Use of a Robot and Flow Injection for Automated Sample Preparation and Analysis of Used Oils by ICP Emission Spectrometry, Spectrochim. Acta, 42B (1987) 169-180.

974.   A.K.S. Ahmad, E.M. Abdel-Moety, A.A. Moustafa and A.E. El-Gendy and E. Ahmed, Flow Injection Analysis of Pharmaceutical Compounds. III. Quantification of Furosemide with Spectrophotometric Detection, Arch. Pharm. Chemi, Sci. Ed., 14 (1986) 113-118.

975.   J. Martinez Calatayud and P. Campins Falco, Spectrophotometric Determination of Chlorhexidine with Bromocresol Green by Flow-Injection and Manual Methods, Anal. Chim. Acta, 189 (1986) 323-328.

976.   I. Lopez Garcia, M. Hernandez Cordoba and C. Sanchez-Pedreno, Flow Injection Atomic Absorption Spectrometry with Air Compensation, Analyst, 112 (1987) 271-276.

977.   D.C. Stone and J.F. Tyson, Models for Dispersion in Flow Injection Analysis. Part 1. Basic Requirements and Study of Factors Affecting Dispersion, Analyst, 112 (1987) 515-521.

978.   J.F. Tyson, Analytical Information from Doublet Peaks in Flow Injection Analysis. Part 1. Basic Equation and Applications to Flow Injection Titrations, Analyst, 112 (1987) 523-526.

979.   A.G. Fogg, C.W. Guta and A.Y. Chamsi, Iodometric and Bromometric Flow Injection Amperometric Methods of Determining Selyte in Which Iodine or Bromine is Monitored at a Glassy Carbon Electrode, Analyst, 112 (1987) 253-258.

980.   S. Motomizu and M. Oshima, Spectrophotometric Determination of Phosphorus as Orthophosphate Based on Solvent Extraction of the Ion Associate of Molybdophosphate with Malachite Green Using Flow Injection, Analyst, 112 (1987) 295-300.

981.   P. Linares, M.D. Luque de Castro and M. Valcarcel, Differential Kinetic Determination of Furfural and Vanillin by Flow Injection Analysis, Microchem. J., 35 (1987) 120-124.

982. S. Dong and P.K. Dasgupta, Fast Fluorometric Flow Injection Analysis of Formaldehyde in Atmospheric Water, Environ. Sci. Technol., 21 (1987) 581-588.

983. J.P. Martinez, M. Gallego and M. Valcarcel, Indirect Atomic Absorption Determination of Chloride by Continuous Precipitation of Silver Chloride in a Flow Injection System, J. Anal. At. Spectrom., 2 (1987) 211-215.

984. H. Bergamin Filho, F.J. Krug, E.A.G. Zagatto, E.C. Arruda and C.A. Coutinho, On-Line Electrolytic Dissolution of Alloys in Flow-Injection Analysis. Part 1. Principles and Application in the Determination of Soluble Aluminum in Steels, Anal. Chim. Acta, 190 (1986) 177-184.

985. N. Ishibashi, T. Imato and K. Tsukiji, Potentiometric and Spectrophotometric Flow-Injection Determinations of Metal Ions with Use of Metal Ion Buffers, Anal. Chim. Acta, 190 (1986) 185-194.

986. J.F. Van Staden, Flow Injection Determination of Inorganic Bromide in Soils with a Coated Tubular Solid-State Bromide-Selective Electrode, Analyst, 112 (1987) 595-599.

987. G.D. Christian and J. Ruzicka, Flow Injection Analysis: a Novel Tool for Plasma Spectroscopy, Spectrochim. Acta, 42B (1987) 157-167.

988. J. Ruzicka, Flow Injection Analysis - A Survey of Its Potential for Continuous Monitoring of Industrial Processes, Anal. Chim. Acta, 190 (1986) 155-163.

989. W.E. Van der Linden, Miniaturization in Flow Injection Analysis. Practical Limitations from a Theoretical Point of View, Trends in Anal. Chem., 6 (1987) 37-40.

990. M. Gisin and C. Thommen, Industrial Process Control by Flow Injection Analysis, Anal. Chim. Acta, 190 (1986) 165-176.

991. P.R. Kraus and S.R. Crouch, Investigation of a Chemiluminescent System for the Determination of Ammonia by Flow-Injection Analysis, Anal. Lett., 20 (1987) 183-200.

992. R. Forteza, V. Cerda, S. Maspoch and M. Blanco, Spectrophotometric Determination of Magnesium(II) by Flow-Injection Analysis Using Succinimide Dioxime, Analusis, 15 (1987) 136-139.

993. T.J. Cardwell, D. Caridi, R.W. Cattrall and I.C. Hamilton, The Spectrophotometric Determination of Iron(III) in a Flow-Injection System with a Mixed Solvent, Anal. Chim. Acta, 192 (1987) 129-132.

994. I.R.C. Whiteside, P.J. Worsfold and A. Lynes, Fluorometric Flow-Injection Determination of Tertiary Amines in Nonaqueous Media, Anal. Chim. Acta, 192 (1987) 77-83.

995. E. Ruiz, M. Hernandez Blanco, E. Lorenzo Abad and L. Hernandez, Determination of Nitrazepam and Flunitrazepam by Flow Injection Analysis Using a Voltammetric Detector, Analyst, 112 (1987) 697-699.

996. D.S. Brown and D.R. Jenke, Determination of Sulfite and Hydrogen Peroxide in Pharmaceutical Matrices via Classical Spectrophotometry and Flow Injection, Analyst, 112 (1987) 899-902.

997. F. Lazaro, M.D. Luque de Castro and M. Valcarcel, Individual and Simultaneous Determination of Ethanol and Acetaldehyde in Wines by Flow Injection Analysis and Immobilized Enzymes, Anal. Chem., 59 (1987) 1859-1863.

998. F.W. Nyasulu and H.A. Mottola, Amperometric Determination of Nitrogen Dioxide in Air Samples by Flow Injection and Reaction at a Gas-Liquid Interface, J. Autom. Chem., 9 (1987) 46-49.

999. C.M. Sakamoto-Arnold and K.S. Johnson, Determination of Picomolar Levels of Cobalt in Seawater by Flow Injection Analysis with Chemiluminescence Detection, Anal. Chem., 59 (1987) 1789-1794.

1000. J.F. Van Staden, On-Line Sulfate Monitoring by Reversed Flow Injection Analysis and Alternating Reagent Injection, Fresenius' Z. Anal. Chem., 326 (1987) 754-756.

1001. K.J. Skogerboe and E.S. Yeung, Stray Light Rejection in Fiber-Optic Probes, Anal. Chem., 59 (1987) 1812-1815.

1002. X. Yang, L. Risinger and G. Johansson, Removal of Humic Acid and Surfactant Interferences in Trace Metal Determinations by Differential-Pulse Anodic Stripping Voltammetry with Use of Adsorption and Chelate Ion-Exchanger Columns in a Flow-Injection System, Anal. Chim. Acta, 192 (1987) 1-8.

1003. M.C. Gutierrez, A. Gomez-Hens and D. Perez-Bendito, A Modular Fluorimetric Stopped-Flow System for Use in Clinical Chemistry, Fresenius' Z. Anal. Chem., 328 (1987) 120-124.

1004. F. Lazaro, M.D. Luque de Castro and M. Valcarcel, Doubly Stopped Flow: A New Alternative to Simultaneous Kinetic Multi Determinations in Unsegmented Flow Systems, Anal. Chem., 59 (1987) 950-954.

1005. J.B. Callis, D.L. Illman and B.R. Kowalski, Process Analytical Chemistry, Anal. Chem., 59 (1987) 624A-635A.

1006. W. Frenzel and G. Schulze, Open Thin Layer Cell - A Flow-through Electrode for Potentiometric Stripping Analysis, Analyst, 112 (1987) 133-136.

1007. D.F. Leclerc, P.A. Bloxham and E. C. Toren, Axial Dispersion in Coiled Tubular Reactors, Anal. Chim. Acta, 184 (1986) 173-185.

1008. P. Petak and K. Stulik, Use of Ion-Selective Electrodes in Industrial Flow Analysis, Anal. Chim. Acta, 185 (1986) 171-178.

1009. S.D. Kolev and E. Pungor, Description of an Axially-Dispersed Plug Flow Model for the Flow Pattern in Elements of Fluid Systems, Anal. Chim. Acta, 185 (1986) 315-319.

1010. H. Hwang and P.K. Dasgupta, Flow Injection Analysis of Trace Hydrogen Peroxide Using an Immobilized Enzyme Reactor, Mikrochim. Acta, 3 (1986) 77-87.

1011. B. Olsson, A Flow Injection System Using Immobilized Peroxidase and Chromogenic Reagents for Possible Determination of Hydrogen Peroxide, Microchem. Acta, 2 (1985) 211-221.

340

1012.   Y. Ikariyama and W.R. Heineman, Polypyrrole Electrode as a Detector for Electroinactive Anions by Flow Injection Analysis, Anal. Chem., 58 (1986) 1803-1806.

1013.   S.W. Willason and K.S. Johnson, A Rapid, Highly Sensitive Technique for the Determination of Ammonia in Seawater, Marine Biol., 91 (1986) 285-290.

1014.   A. Brezezinska-Pandyn, J. Van Loon and R. Hancock, Comparison of the Determination of Arsenic in Environmental Samples by Different Analytical Techniques, At. Spectrosc., 7 (1986) 72-75.

1015.   C.F. Mascone, Flow Injection Analysis Moves into CPI Plants, Chemical Engineering, 7 (1986) 14-15.

1016.   S.R. Bysouth and J.F. Tyson, Flow Manifold for Automated On-Line Dilution of Standards for Flame Atomic Absorption Spectrometry and Its Use in a Null Measurement Method, J. Anal. At. Spectrom., 2 (1987) 217-220.

1017.   A. Eaton, Literature Review Issue. Chemical Analysis. Inorganics, J. Water Pollut. Control Fed., 59 (1987) 313-328.

1018.   A Tanaka, K. Obata and T. Deguchi, Fluorimetric Catalytic Determination of Iodide by Flow Injection Analysis, Anal. Sci., 2 (1986) 197-198.

1019.   H.A. Mottola, Enzymes as Analytical Reagents: Substrate Determinations with Soluble and with Immobilized Enzyme Preparations, Analyst, 112 (1987) 719-727.

1020.   M. Valcarcel, Selectivity and Kinetics in Analytical Chemistry, Analyst, 112 (1987) 729-737.

1021.   A. Fernandez, M.D. Luque de Castro and M. Valcarcel, Flow Injection System for Kinetic Determinations Based on the Use of Two Serial Injection Valves, Analyst, 112 (1987) 803-807.

1022.   N.P. Evrimidis, Effect of Hydroxylamine on Chemiluminescence Intensity Generated During the Oxidation of Pyrogallol with Periodate, Analyst, 112 (1987) 825-829.

1023.   S.A. Al-Tamrah, A. Townshend and A.R. Wheatley, Flow Injection Chemiluminescence Determination of Sulphite, Analyst, 112 (1987) 883-886.

1024.   A.G. Fogg and R.M. Alonso, Oxidative Amperometric Flow Injection Determination of Cyanide at an Electrochemically Pre-Treated Glassy Carbon Electrode, Analyst, 112 (1987) 1071-1072.

1025.   J. Toei, Use of Stirred Chamber to Increase the Efficiency of Solid-Liquid Reactions in Flow Injection Analysis. Part I. Determination of Sulphate Using Barium Chloranilate, Analyst, 112 (1987) 1067-1069.

1026.   C. De Ruiter, J.H. Wolf, U.A.T. Brinkman and R.W. Frei, Design and Evaluation of a Sandwich Phase Separator for On-Line Liquid/Liquid Extraction, Anal. Chim. Acta, 192 (1987) 267-275.

1027.   C.L.M. Stults, A.P. Wade and S.R. Crouch, Experimental Studies on the Effect of Temperature on Dispersion in a Flow-Injection System, Anal. Chim. Acta, 192 (1987) 301-308.

1028. T.P. Tougas and C.Y. Yuan, Simultaneous Determination of Multiple Components in Flow-Injection Systems by Square-Wave Amperometry, Anal. Chim. Acta, 192 (1987) 327-331.

1029. L.R. Silva, L.E. Leon and A.C. Calvo, Indirect Determination of Iron in a Flow-Injection System with Amperometric Detection, Anal. Chim. Acta, 192 (1987) 349-353.

1030. C.L.M. Stults, A.P. Wade and S.R. Crouch, Computer-Assisted Optimization of an Immobilized-Enzyme Flow-Injection System for the Determination of Glucose, Anal. Chim. Acta, 192 (1987) 155-163.

1031. M. Yamamoto, K. Takada, T. Kumamaru, M. Yasuda, S. Yokoyama and Y. Yamamoto, Membrane Gas-Liquid Separator for Flow Injection Hydride-Generation Atomic Absorption Spectrometry, Anal. Chem., 59 (1987) 2446-2448.

1032. P. Martinez-Jimenez, M. Gallego and M. Valcarcel, Pre-Concentration and Determination of Trace Amounts of Lead in Water by Continuous Precipitation in an Unsegmented-Flow Atomic Absorption Spectrometric System, Analyst, 112 (1987) 1233-1236.

1033. G. Knapp, K. Muller, M. Strunz and W. Wegscheider, Automation in Element Pre-Concentration with Chelating Ion Exchangers, J. Anal. At. Spectrom., 2 (1987) 611-614.

1034. C. McLeod, Flow Injection Techniques in Inductively Coupled Plasma Spectrometry, J. Anal. At. Spectrom., 2 (1987) 549-552.

1035. A.G. Cox, C.W. McLeod, D.L. Miles and J. Cook, Trace Enrichment and Determination of Sulphate by Flow Injection Inductively Coupled Plasma Atomic Emission Spectrometry, J. Anal. At. Spectrom., 2 (1987) 553-555.

1036. L. Nord, K. Backstrom, L.-G. Danielsson, F. Ingman and B. Karlberg, Extraction Rate in Liquid-Liquid Segmented Flow Injection Analysis, Anal. Chim. Acta, 194 (1987) 221-233.

1037. J.B. Noffsinger and N.D. Danielson, Generation of Chemiluminescence upon Reaction of Aliphatic Amines with Tris(2,2'-bipyridine)ruthenium(III), Anal. Chem., 59 (1987) 865-868.

1038. S. Motomizu, M. Oshima and A. Hirashima, Spectrophotometric Determination of Phosphorus in River Water Based on the Reaction of Vanado-Molybdophosphate with Malachite Green, Anal. Chim Acta, 211 (1988) 119-127.

1039. N. Ishibashi, T. Imato and K. Tsukiji, Potentiometric and Spectrophotometric Flow-Injection Determination of Metal Ions with Use of Metal Ion Buffers, Anal. Chim. Acta, 190 (1986) 185-194.

1040. J.F. Van Staden, Flow Injection Determination of Inorganic Bromide in Soils with a Coated Tubular Solid-State Electrode, Analyst, 112 (1987) 595-599.

1041. P.O. Scokart, K. Meeus-Verdinne and R. De Borger, Speciation of Heavy Metals in Polluted Soils by Sequential Extraction and ICP Spectrometry, Int. J. Environ. Anal. Chem., 29 (1987) 305-315.

1042. M. Valcarcel and M.D. Luque de Castro, Continuous Separation Techniques in Flow Injection Analysis, A Review, J. Chromatogr., 393 (1987) 3-23.

1043. T. Takeuchi, Y. Kabasawa, R. Horikawa and T. Tanimura, Flow Injection Determination of Drugs by Specific Detection of Carboxylic Acids, Analyst, 113 (1988) 1673-1675.

1044. M. Blanco, J. Gene, H. Iturriaga and S. Maspoch, Application of a Photodiode Array Detector to Multicomponent Determination by Flow Injection Analysis, Analyst, 112 (1987) 619-622.

1045. K. Uchida, D. Yoshizawa, M. Tomoda and S. Saito, Determination of Glucose by Flow-Injection Analysis with Merging-Zone Technique, Anal. Sci., 2 (1987) 181-183.

1046. G.G. Neuberger and D.C. Johnson, Pulsed Colorimetric Detection of Carbohydrates at a Constant Detection Potential at Gold Electrodes in Alkaline Media, Anal. Chim. Acta, 192 (1987) 205-213.

1047. B. Karlberg, The Theoretical and Practical Development of Flow Injection Extraction, Anal. Chim. Acta, 214 (1988) 29-39.

1048. A. Fernandez, M.D. Luque de Castro and M. Valcarcel, Voltammetric-Enzyme Determination of Ethanol in Whole Blood by Flow Injection Analysis, Fresenius' Z. Anal. Chem., 327 (1987) 552-554.

1049. J.F. Van Staden, On-Line Sulphate Monitoring by Reversed Flow Injection Analysis and Alternting Reagent Injection, Fresenius' Z. Anal. Chem., 326 (1987) 754-756.

1050. S. Motomizu, H. Mikasa and K. Toei, Fluorimetric Determination of Nitrate in Natural Waters with 3-Amino-1,5-naphtalene-disulfonic Acid in a Flow Injection System, Anal. Chim. Acta, 193 (1987) 343-347.

1051. N. Ohno and T. Sakai, Spectrophotometric Determination of Iron in Boiler and Well Waters by Flow Injection Analysis Using 2-Nitroso-5-(N-propyl-N-sulfopropylamino)phenol, Analyst, 112 (1987) 1127-1130.

1052. K. Oguma, K. Nishiyama and R. Kuroda, Spectrophotometric Flow Injection Analysis of Silicates for Manganese, Anal. Sci., 3 (1987) 251-255.

1053. C. De Ruiter, J.H. Wolf, U.A.T. Brinkman and R.W. Frei, Design and Evaluation of a Sandwich Phase Separator for On-Line Liquid/Liquid Extraction, Anal. Chim. Acta, 192 (1987) 267-275.

1054. C.L.M. Stults, A.P. Wade and S.R. Crouch, Immobilized Enzyme Kinetic Study of D-Glucose Mutarotation by Flow Injection Analysis, Anal. Chem., 59 (1987) 2245-2247.

1055. J. Fucsko, K. Toth and E. Pungor, J. Kunovits and H. Puxbaum, Application of Ion-Selective Electrodes in Environmental Analysis. Determination of Acid and Fluoride Concentrations in Rainwater with a Flow-Injection System, Anal. Chim. Acta, 194 (1987) 163-170.

1056. J. Toei, Use of a Stirred Chamber to Increase the Efficiency of Solid-Liquid Reactions in Flow-Injection Analysis. Part 1. Determination of Sulfate Using Barium Chloranilate, Analyst, 112 (1987) 1067-1069.

1057. A.G. Fogg and R.M. Alonso, Oxidative Amperometric Flow-Injection Determination of Cyanide at an Electrochemically Pretreated Glassy Carbon Surface, Analyst, 112 (1987) 1071-1072.

1058. W.A. De Oliveira and A.S. Mendes, Determination of Calcium and Magnesium in Limestone and Dolomite by Enthalpimetric Flow-Injection Analysis, Talanta, 34 (1987) 543-546.

1059. A. Trojanik and S. Bruckenstein, Dual Porous Electrode Membrane Cell for Detection of Nonelectroactive Species in Flowing Streams, Anal. Chem., 59 (1987) 2260-2263.

1060. B. Bermudez, F. Lazaro, M.D. Luque de Castro and M. Valcarcel, Individual and Simultaneous Fluorimetric Determination of Glycine and Cysteine by Flow Injection Analysis, Microchem. J., 35 (1987) 315-320.

1061. J.S. Canham and G.E. Pacey, Automated Free Fatty Acid Determination Using Flow Injection Analysis Solvent Extractions, J. Am. Oil Chem. Soc., 64 (1987) 1004-1007.

1062. M. Yamada and S. Suzuki, Cyclic Flow-Injection Determination of Copper with Hexadecyltrimethylammonium Bromide Micelle-Enhanced, Fluorescein-Sensitized Chemiluminescence Detection, Anal. Chim. Acta, 193 (1987) 337-341.

1063. Y. Narusawa and T. Hashimoto, Simultaneous Determination of Phosphate, Silicate and Arsenate by On-Line Column Flow Injection Analysis, Chem. Lett., 7 (1987) 1367-1370.

1064. N. Ohyabu, M. Fujimura, K. Tanimizu, Y. Okuno, M. Tabata and T. Murachi, 'One-Shot' Flow Injection Analysis with Immobilized Enzyme Columns: Clinical Applications, Anal. Sci., 3 (1987) 277-278.

1065. F. Canete, A. Rios, M.D. Luque de Castro and M. Valcarcel, Flow-Injection Cyclic Voltammetry, Anal. Chim. Acta, 211 (1988) 287-292.

1066. Y. Onari, Application of the Flow Injection Analysis Based on Iodometry to the Determination of Ozone Decomposition Rate, Bull. Chem. Soc. Jpn., 60 (1987) 3074-3076.

1067. E.A.G. Zagatto, O. Bahia Filho and H. Bergamin Filho, Recording the Real Sample Distribution and Concentration/Time Functions in Flow Injection Analysis, Anal. Chim. Acta, 193 (1987) 309-314.

1068. C.D. Crowe, H.W. Levin, D. Betteridge and A.P. Wade, A Random-Walk Simulation of Flow-Injection Systems with Merging Zones, Anal. Chim. Acta, 194 (1987) 49-60.

1069. A.L. Plant, L. Locascio-Brown, M.V. Brizgys and R.A. Durst, Liposome-Enhanced Flow Injection Immunoanalysis, Biotechnology, 6 (1988) 266-269.

1070. C. Pasquini and L. Cardoso de Faria, Flow-Injection Determination of Ammonia in Kjeldahl Digests by Gas Diffusion and Conductometry, Anal. Chim. Acta, 193 (1987) 19-27.

1071. A. Fernandez, M.D. Luque de Castro and M. Valcarcel, Formation of Two Reaction Zones in Flow-Injection Systems for Kinetic Determination of Cobalt and Nickel, Anal. Chim. Acta, 193 (1987) 107-118.

1072. A. Hulanicki, W. Matuszewski and M. Trojanowicz, Flow-Injection Determination of Nitrite and Nitrate with Biamperometric Detection at Two Platinum Wire Electrodes, Anal. Chim. Acta, 194 (1987) 119-127.

1073.   W. Matuszewski, A. Hulanicki and M. Trojanowicz, Flow-Injection Single-Point Titration of Acids with Biamperometric Detection at Polarized Platinum Electrodes, Anal. Chim. Acta, 194 (1987) 269-274.

1074.   J.M. Martin and P.J. Ihrig, Quantitation of Metals in Liquid Samples by Computer Intelligent Flow Injection Inductively Coupled Plasma Emission Spectrometry, Appl. Spectrosc., 41 (1987) 986-993.

1075.   S. Motomizu, S.C. Rui, M. Oshima and K. Toei, Spectrophotometric Determination of Trace Amounts of Nitrite Based on the Nitrosation Reaction with N,N-Bis(2-hydroxypropyl)-aniline and Its Application in Flow Injection Analysis, Analyst, 112 (1987) 1261-1263.

1076.   G. Jonsson and L. Gorton, An Amperometric Glucose Electrode Based on Adsorbed Glucose Oxidase on Palladium/Gold Modified Graphite, Anal. Lett., 20 (1987) 839-855.

1077.   J.M. Fernandez-Romeo, M.D. Luque de Castro and M. Valcarcel, Determination of Total Cholesterol in Serum by Flow Injection Analysis with Immobilized Enzymes, Clin. Chim. Acta, 167 (1987) 97-104.

1078.   S. Motomizu, Y. Hazaki, M. Oshima and K. Toei, Spectrophotometric Determination of Anionic Surfactants in River Water with Cationic Azo Dye by Solvent Extraction-Flow Injection Analysis, Anal. Sci., 3 (1987) 265-269.

1079.   A. Farran and J. De Pablo, Evaluation of Combined Flow Injection High Performance Liquid Chromatography for the Determination of Three Organophosphorus Pesticides in Liquid Wastes, Int. J. Environ. Anal. Chem., 30 (1987) 59-68.

1080.   G.E. Pacey and B.P. Bubnis, Flow Injection Analysis: Beyond Laboratory Curiosity Stage, Trends in Anal. Chem., 6 (1987) 165-168.

1081.   L. Ilcheva, M. Trojanowicz and T.K.V. Krawczyk, Effect of Addition of Main Ion to Carrier Solution in Potentiometric Flow-Injection Measurements with Solid State Ion-Selective Electrodes, Fresenius' Z. Anal. Chem., 328 (1987) 27-32.

1082.   T. Murachi and M. Tabata, Use of a Bioreactor Consisting of Sequentially Aligned L-Glutamate Dehydrogenase and L-Glutamate Oxidase for the Determination of Ammonia by Chemiluminescence, Biotechnol. Appl. Biochem., 9 (1987) 303-309.

1083.   C.W. McLeod, Flow Injection Techniques in Inductively Coupled Plasma Spectrometry. Plenary Lecture, J. Anal. At. Spectrom., 2 (1987) 549-552.

1084.   A. Gustafsson and O. Nygren, Characterization of a Nebulizer Interface for Flame Atomic Absorption Spectroscopy, Spectrochim. Acta, 42B (1987) 883-888.

1085.   G. Schulze, M. Koschany and O. Elsholz, A Flow Cell with Flexible Deposition Efficiency for a Dual-Detection System Based on Potentiometric Stripping Analysis and Atomic Absorption Spectrometry, Anal. Chim. Acta, 196 (1987) 153-161.

1086.   J.F. Van Staden, On-Line Quality Control in Concentrated Hydrochloric Acid Production Plants. Flow Injection Determination of Hydrochloric Acid Content in Concentrated Hydrochloric Acid by Atomated Prevalve Dilution and a Coated Tubular Solid-State Chloride-Selective Electrode, Fresenius' Z. Anal. Chem., 328 (1987) 68-70.

1087.   J. Pawliszyn, Detection of Flowing Samples with a Selective Concentration Gradient Method, Anal. Chem., 60 (1988) 766-773.

1088.   A.A. Alwarthan and A. Townshend, Chemiluminescence Determination of Iron (II) and Titanium (III) by Flow-Injection Analysis Based on Reactions with and without Luminol, Anal. Chim. Acta, 196 (1987) 135-140.

1089.   A.T. Senior and J.D. Glennon, Use of Acetohydroxamic Acid in the Direct Spectrophotometric Determination of Iron (III) and Iron (II) by Flow-Injection Analysis, Anal. Chim. Acta, 196 (1987) 333-336.

1090.   G.N. Eccles and W.C. Purdy, Pulse Cyclic Voltammetry. II. Flowing Solutions, Can. J. Chem., 65 (1987) 1795-1799.

1091.   J.J. Laserna, A. Berthold and J.D. Winefordner, Quantitative Analysis by Surface-Enhanced Raman Spectrometry on Silver Hydrosols in a Flow-Injection System, Talanta, 34 (1987) 745-747.

1092.   U.W. De Alwis, B.S. Hill, B.I. Meiklejohn and G.S. Wilson, Reversibly Immobilized Glucose Oxidase in the Amperometric Flow-Injection Determination of Glucose, Anal. Chem., 59 (1987) 2688-2691.

1093.   J. Alonso, J. Bartroli, J. Coello and M. Del Valle, Simultaneous Optimization of Variables in FIA Systems by Means of the Simplex Method, Anal. Lett., 20 (1987) 1247-1263.

1094.   A.S. Attiyat, Sample Solvent/Carrier Combination for Flow Injection Analysis-Atomic Absorption Spectrometry, Anal. Chem., 4 (1987) 26-35.

1095.   B.A. Woods, J. Ruzicka and G.D. Christian, Sequential Determination of Both Acids and Bases by Optosensing Flow Injection Analysis Using a Single-Line Manifold, Anal. Chem., 59 (1987) 2767-2773.

1096.   M.R. Pereiro Garcia, M.E. Diaz Garcia and A. Sanz Medel, Flow Injection Ion-Exchange Preconcentration for the Determination of Aluminum by Atomic Absorption Spectrometry and Inductively Coupled Plasma Atomic Emission Spectrometry, J. Anal. At. Spectrom., 2 (1987) 699-703.

1097.   U.W. De Alwis and G.S. Wilson, Rapid Heterogeneous Competitive Electrochemical Immunoassay for IgG in the Picomole Range, Anal. Chem., 59 (1987) 2786-2789.

1098.   Y. Suzuki, N. Arai and M. Okamoto, Flow Injection Analysis of Feed and Premix for Monensin and Salinomycin, Anal. Sci., 3 (1987) 441-444.

1099.   S. Motomizu, K. Toei, T. Kuwaki and M. Oshima, Gas-Diffusion Unit with Tubular Microporous Poly(tetrafluoroetylene) Membrane for Flow-Injection Determination of Carbon Dioxide, Anal. Chem., 59 (1987) 2930-2932.

1100.   Y. Hayashi, K. Zaitsu and Y. Ohkura, Assay for Guanase in Blood Serum by Flow Injection Analysis with Fluorescence Detection, Anal. Chim. Acta, 197 (1987) 51-56.

1101. J.M. Fernandez-Romero, M.D. Luque de Castro and M. Valcarcel, Enzymic Determination of Total Cholesterol in Serum by Flow Injection Analysis, J. Pharm. Biomed. Anal., 5 (1987) 333-340.

1102. H. Ukeda, S. Tanaka, K. Matsumoto and Y. Osajima, Amperometric Flow Injection Measurement of Protein in Diary Products Using Glutaraldehyde, Agric. Biol. Chem., 51 (198x) 2835-2836.

1103. P.W. Alexander and J. Koopetngarm, Flow-Injection Determination of Phosphate Species in Detergents with a Calcium Ion-Selective Electrode, Anal. Chim. Acta, 197 (1987) 353-359.

1104. D.J. Roberts and K.V. Kaholola, Flow Injection Carbon Skeleton Gas Chromatography, Proc. Anal. Div. Chem. Soc., 23 (1987) 437-439.

1105. P.K. Dasgupta, L.S. Rhee and E.L. Loree, A Simple and Versatile Photodetector System for Peak Width Measurement-Based Flow Injection Analysis, Spectroscopy, 2 (1987) 39-43.

1106. A. Maquieira, M.D. Luque de Castro and M. Valcarcel, Determination of Reducing Sugars in Wine by Flow Injection Analysis, Analyst, 112 (1987) 1569-1572.

1107. M.P. Keuken, F.P. Bakker, W.A. Lingerak and J. Slanina, Flow Injection Analysis of Hydrogen Peroxide, Sulfite, Formaldehyde and Hydroxymethanesulfonic Acid in Precipitation Samples, Int. J. Environ. Anal. Chem., 31 (1987) 263-279.

1108. J.R. Gord, G. Gordon and G.E. Pacey, Selective Chlorine Determination by Gas-Diffusion Flow Injection Analysis with Chemiluminescent Detection, Anal. Chem., 60 (1988) 2-4.

1109. J.F. Van Staden and C.C.P. Wagener, Flow-Injection Analysis with a Coated Tubular Solid-State Copper(2)-Selective Electrode, Anal. Chim. Acta, 197 (1987) 217-227.

1110. D. Yoshizawa, M. Tomoda, K. Uchida, K. Fukushima and S. Saito, Data Processing for Flow Injection Analysis Using Personal Computer of MSX Standard System, Anal. Sci., 3 (1987) 467-468.

1111. B.S. Hui and C.O. Huber, Temperature Effects on Amperometric Detection at Nickel Oxide Electrodes in Flow-Injection Systems, Anal. Chim. Acta, 197 (1987) 361-365.

1112. T. Kakizaki, K. Hasebe and H. Yoshida, Catalytic-Polarographic Determination of Vanillylmandelic Acid in Urine Using a Continuous Deoxygenation-Flow Injection System, Analyst, 112 (1987) 1593-1596.

1113. E.A.G. Zagatto, B.F. Reis, M. Martinelli, F.J. Krug, H. Bergamin Filho and M.F. Gine, Confluent Streams in Flow Injection Analysis, Anal. Chim. Acta, 198 (1987) 153-163.

1114. W.E. Bauer, A.P. Wade and S.R. Crouch, Peak Readout Device for Transient Signals from Flow Injection Analysis, Anal. Chem., 60 (1988) 287-288.

1115. A. Al-Wehaid and A. Townshend, Flow-Injection Amplification for the Spectrophotometric Determination of Iodide, Anal. Chim. Acta, 198 (1987) 45-51.

1116. K.P. Ang, H. Gunasingham, B.T. Tay, V.S. Herath, P.Y. Teo, P.C. Thiak, B. Kuah and K.L. Tan, Determination of Glucose Using Flow Injection with a Carbon Fiber Based Enzyme Reactor, Analyst, 112 (1987) 1433-1435.

1117. J. Ruz, M.D. Luque de Castro and M. Valcarcel, Flow Injection Determination of Ethanol in Whole Blood Using Immobilized Enzymes, Microchem. J., 36 (1987) 316-322.

1118. P. Linares, J. Ruz, M.D. Luque de Castro and M. Valcarcel, Enzymatic Determination of Ethanol in Saliva by Flow Injection Analysis, J. Pharm. Biomed. Anal., 5 (1987) 701-706.

1119. S. Yonezawa, F. Morishita and T. Kojima, Simultaneous Determination of Triglycerides and ß-D-Glucose by Flow Injection Analysis Using Immobilized Enzyme Open Tubular Reactors, Anal. Sci., 3 (1987) 553-556.

1120. A. Maquieira, M.D. Luque de Castro and M. Valcarcel, Simultaneous Enzymatic Determination of Methanol and Ethanol by Flow Injection Analysis, Microchem. J., 36 (1987) 309-315.

1121. K. Matsumoto, H. Kamikado, H. Matsubara and Y. Osajima, Simultaneous Determination of Glucose, Fructose, and Sucrose in Mixtures by Amperometric Flow Injection Analysis with Immobilized Enzyme Reactors, Anal. Chem., 60 (1988) 147-151.

1122. D.R. Turner, S. Knox, M. Whitfield and M. Correia dos Santos, Flow Injection Titration of Alkalinity in Natural Waters, Anal. Proc., 24 (1987) 360-362.

1123. Y. Zhang, P. Riby, A.G. Cox, C.W. MacLeod, A.R. Date and Y.Y. Cheung, On-Line Preconcentration and Determination of Lead in Potable Water by Flow Injection Atomic Absorption Spectrometry, Analyst, 113 (1988) 125-128.

1124. A. Rios, M.D. Luque de Castro and M. Valcarcel, Determination of Viscosity with an Open-Closed Flow Injection System, Talanta, 34 (1987) 915-919.

1125. Y. Ikariyama, C. Galiatsatos, W.R. Heineman and S. Yamauchi, Polypyrrole Electrode as a Detector of Anionic Substances, Sens. Actuators, 12 (1987) 455-461.

1126. H. Xiaohua, M.J. Gordon and R.N. Zare, Bias in Quantitative Capillary Zone Electrophoresis Caused by Electrokinetic Sample Injection, Anal. Chem., 60 (1988) 375-377.

1127. T. Ohta, Y. Arai and S. Takitani, Fluorometric Flow-Injection Analysis of Saliva for Nitrite, Anal. Sci., 3 (1987) 549-551.

1128. L.J. Blacik, H.G. Weinstein and M. Breen, Automated Kinetic Enzyme Assay for Sodium/Potassium ATPase by Flow Injection Analysis, Biochem. Arch., 3 (1987) 371-381.

1129. R.Q. Thompson, K. Hidong and C.E. Miller, Comparison of Immobilized Enzyme Reactors for Flow Injection Systems, Anal. Chim. Acta, 198 (1987) 165-172.

1130. J.F. Tyson, The Principle of Flow Injection Analysis, Proc. Anal. Div. Chem. Soc., 24 (1987) 362-364.

348

1131. M. Trojanowicz and W. Frenzel, Flow Injection Potentiometry for Low Level Measurements in the Presence of Sensed Ion in the Carrier, Fresenius' Z. Anal. Chem., 328 (1987) 653–656.

1132. J. Martinez Calatayud, R. Mico Albert and P. Campico, Flow Injection Manifold for Simultaneous Spectrophotometric Determination of Bismuth(III) and Lead(II), Anal. Lett., 20 (1987) 1379–1398.

1133. W.R. Blair, E.J. Parks, G.J. Olson, F.E. Brinckham, M.C. Valeiras-Price and J.M. Bellama, Characterization of Organotin Species Using Microbore and Capillary Liquid Chromatographic Techniques with an Epifluorescence Microscope as a Novel Imaging Detector, J. Chromatogr., 410 (1987) 383–394.

1134. A.M. Alumaibed and A. Townshend, Determination of Acetaldehyde by Flow Injection Analysis with Soluble or Immobilized Aldehyde Dehydrogenase, Anal. Chim. Acta, 198 (1987) 37–44.

1135. K. Zaitsu, M. Nakayama and Y. Ohkura, Sensitive Flow Injection Determination of L-Lactate in Human Blood with Immobilized Enzyme Columns and Fluorimetric Detection, Anal. Chim. Acta, 201 (1987) 351–355.

1136. E. Chico Guijarro, P. Yanez-Sedeno and L.M. Polo Diez, Determination of Paraquat by Flow-Injection Spectrophotometry, Anal. Chim. Acta, 199 (1987) 203–208.

1137. M.A.Z. Arruda and E.A.G. Zagatto, A Simple Stopped Flow Method with Continuous Pumping for the Spectrophotometric Flow-Injection Determination of Boron in Plants, Anal. Chim. Acta, 199 (1987) 137–145.

1138. K.S. Johnson, C.M. Sakamoto-Arnold, S.W. Willason and C.L. Beehler, Reagent Injection Flow Analysis: Application to the Determination of Nanomolar Levels of Hydrogen Peroxide in Seawater, Anal. Chim. Acta, 201 (1987) 83–94.

1139. J. Moller and M. Martin, Determination of Phenol in Water by Flow Injection Analysis, Fresenius' Z. Anal. Chem., 329 (1988) 728–731.

1140. J. F. Van Staden, Advances in Flow Injection Analysis with Coated Tubular Ion-Selective Electrode Units, Proc. Anal. Div. Chem. Soc., 24 (1987) 331–333.

1141. A. Rios, F. Lazaro, M.D. Luque de Castro and M. Valcarcel, Multidetection Flow-Injection Techniques for Manipulation of Sensitivity, Amplification and Dilution Methods, Anal. Chim. Acta, 199 (1987) 15–27.

1142. J.B.F. Lloyd, Anodic Response to Oxygen of an Electrochemical Detector in High-Performance Liquid Chromatography and Flow-Injection Analysis, Anal. Chim. Acta, 199 (1987) 161–165.

1143. J. Alonso, J. Bartroli, M. Del Valle, M. Escalada and R. Barber, Sandwich Techniques in Flow-Injection Analysis, Part 1. Continuous Recalibration Techniques for Process Control, Anal. Chim. Acta, 199 (1987) 191–196.

1144. M. Blanco, J. Gene, H. Iturriaga, S. Maspoch and J. Riba, Diode-Array Detectors in Flow-Injection Analysis, Talanta, 34 (1987) 987–993.

1145. M.H. Memon and P.J. Worsfold, Use of Microemulsions in Flow-Injection Analysis: Spectrophotometric Determination of Copper, Anal. Chim. Acta, 201 (1987) 345–350.

1146. C. Gooijer, R.A. Baumann and N.H. Velthorst, The Potential of Liquid State Molecular Phosphorescence in Analytical Chemistry, Prog. Anal. Spectrosc., 10 (1987) 573-599.

1147. Y. Hayashi, K. Zaitsu and Y. Ohkura, Assay of Purine Nucleoside Phosphorylase in Erythrocytes by Flow-Injection Analysis with Fluorescence Detection, Chem. Pharm. Bull., 35 (1987) 4574-4578.

1148. C.J. Dowle, B.G. Cooksey and J.M. Ottaway, Determination of Ionic Surfactants by Flow Injection Pseudotitration, Analyst, 113 (1988) 117-119.

1149. J. Toei, Multifunction Valve for Flow Injection Analysis, Anal. Lett., 20 (1987) 1951-1976.

1150. Yu.A. Zolotov, L.K. Shpigun, I.Ya. Kolotyrrkina, E.A. Novikov and O.V. Bazanova, Trace Determination of Some Heavy Metals in Waters by Flow-Injection Spectrophotometry and Potentiometry, Anal. Chim. Acta, 200 (1987) 21-33.

1151. B.A. Woods, J. Ruzicka, G.D. Christian, N.J. Rose and R.J. Charlson, Measurement of Rainwater pH by Optosensing Flow Injection Analysis, Analyst, 113 (1988) 301-306.

1152. C. Latorre, M. Hernandez Blanco, E.L. Abad, J. Vicente and L. Hernandez, Determination of Clonazepam by Flow Injection Analysis, Analyst, 113 (1988) 317-319.

1153. E.H. Hansen, Exploitation of Gradient Techniques in Flow Injection Analysis, Fresenius' Z. Anal. Chem., 329 (1988) 656-659.

1154. J.F. Tyson, Flow Injection Calibration Techniques, Fresenius' Z. Anal. Chem., 329 (1988) 663-667.

1155. R.E. Sioda and D.J. Curran, Flow Phenomena in Flow Injection Analysis and Flow Electrolysis, J. Electroanal. Chem. Interfacial Electrochem., 239 (1988) 1-7.

1156. S.D. Kolev and E. Pungor, Unsteady Motion in Single-Line Flow-Injection Systems, Anal. Chim. Acta, 201 (1987) 109-116.

1157. W. Frenzel, Potential of Modified Reverse Flow Injection Analysis for Continuous Monitoring and Process Control, Fresenius' Z. Anal. Chem., 329 (1988) 668-674.

1158. J.F. Tyson, A Simple Lecture Demonstration of Flow Injection Analysis, Fresenius' Z. Anal. Chem., 329 (1988) 675-677.

1159. W.E. Van der Linden, M. Box, H.H. Heskamp and H. Wilms, Some Applications of Enthalpimetric Detection in Flow Injection Analysis, Fresenius' Z. Anal. Chem., 329 (1987) 440-443.

1160. A.S. Attiyat, Comparative Study of Solvent Systems for Liquid Carriers and Sample Solvents in Flow Injection Analysis-Atomic Absorption Spectrometry in Measurements of Zinc, Can. J. Spectrosc., 32 (1987) 118-125.

1161. K.H. Al-Sowdani and A. Townshend, Flow-Injection Determination of Europium after On-Line Reduction, Anal. Chim. Acta, 201 (1987) 339-343.

1162.   H. Mori and K. Natsume, Flow Injection-Fluorimetric Determination of Amino Acids Utilizing Enhanced Catalytic Reaction, Anal. Sci., 3 (1987) 581-582.

1163.   C. Liang, P. Vacha and W.E. Van der Linden, Determination of Water in Organic Solvents by Flow-Injection Analysis with Karl Fischer Reagent and a Biamperometric Detection System, Talanta, 35 (1988) 59-61.

1164.   J. Abdul Hamid, G.J. Moody and J.D.R. Thomas, Chemically Immobilized Tri-Enzyme Electrode for the Determination of Sucrose Using Flow Injection Analysis, Analyst, 113 (1988) 81-85.

1165.   B.A. Petersson, H.B. Andersen and E.H. Hansen, Determination of Urea in Undiluted Blood Samples by Flow Injection Analysis Using Optosensing, Anal. Lett., 20 (1987) 1977-1994.

1166.   J. Toei, Rapid, Miniaturized Procedure for the Determination of Glucose Based on Flow Injection Principles, Analyst, 112 (1987) 1565-1568.

1167.   J. Toei, Determination of Glucose in Clinical Samples by Flow Reversal Flow Injection Analysis, Analyst, 113 (1988) 475-478.

1168.   J.M. Hungerford and G.D. Christian, Chemical Kinetics with Reagent Dispersion in Single-Line Flow-Injection Systems, Anal. Chim. Acta, 200 (1987) 1-19.

1169.   B. Koch, Determination of Sulphate by a Modified Flow-Injection Analysis Method and Comparison with the Method DIN 38405 Part 5, Fresenius' Z. Anal. Chem., 329 (1988) 707-710.

1170.   M.A. Hernandez Torres, M.G. Khaledi and J.G. Dorsey, Micellar-Catalyzed Reactions for Flow-Injection Systems. Determination of Pyridoxal, Anal. Chem. Acta, 201 (1987) 67-76.

1171.   W. Frenzel, Ion-Sensitive Electrodes in Flow-Injection Analysis - Improved Performance and Unique Applications, Fresenius' Z. Anal. Chem., 329 (1988) 698-700.

1172.   L. Bezur, Impulse Nebulization Atomic Absorption Spectrometry. Part A. Model for the Response of a Flame Atomizer, J. Anal. At. Spectrom., 3 (1988) 217-225.

1173.   P. Linares, F. Lazaro, M.D. Luque de Castro and M. Valcarcel, Effects of Ultrasonic Irradiation in Flow-Injection Systems, Anal. Chim. Acta, 200 (1987) 51-91.

1174.   G.J. Moody, J.M. Slater and J.D.R. Thomas, Membrane Design and Photocuring Encapsulation of Flatpack Based Ion-Sensitive Field-Effect Transistors, Analyst, 113 (1988) 103-108.

1175.   J.F. Tyson and S.R. Bysouth, Network Flow Injection Manifolds for Sample Dilution and Calibration in Flame Atomic Absorption Spectrometry, J. Anal. At. Spectrom., 3 (1988) 211-215.

1176.   J. Moller, New Injection Valves and Their Application in Flow Injection Analysis, Fresenius' Z. Anal. Chem., 329 (1988) 685-687.

1177.   J.C. De Andrade and E.M. Loboschi, Simple Digital Counter or Use with Monosegmented Continuous Flow Analysis and Related Techniques, Analyst, 113 (1988) 295-296.

1178. Z. Fang, S. Xu and S. Zhang, Fundamental and Practical Considerations in the Design of On-Line Column Preconcentration for Flow-Injection Atomic Spectrometric Systems, Anal. Chim. Acta, 200 (1987) 35-49.

1179. U. Sundqvist, A New Microdialysis Unit for Flow Injection Analysis, Fresenius' Z. Anal. Chem., 329 (1988) 688-690.

1180. K. Cammann, Flow Injection Analysis with Electrochemical Detection, Fresenius' Z. Anal. Chem., 329 (1988) 691-697.

1181. G. Schulze, M. Brodowsky, O. Elsholz and A. Thiele, Dual Detection for Optimization of Gas Diffusion in Flow Injection Analysis. Determination of Ammonium and Sulphide, Fresenius' Z. Anal. Chem., 329 (1988) 714-717.

1182. W.T. Kok, D.T. Thuy, T.V. Nghi and G. Den Boef, The Application of Strongly Reducing Agents in Flow Injection Analysis. Part 6. Molybdenum (III), Anal. Chim. Acta, 200 (1987) 533-538.

1183. A. Nabi and P.J. Worsfold, Determination of Hydrogen Peroxide and Cobalt(II) by Flow-Injection Analysis with Chemiluminescence Detection, J. Chem. Soc. Pak., 9 (1987) 575-579.

1184. S. Sakura and H. Imai, Determination of Sub-nmol Hydrogen Peroxide by Electrochemiluminescence of Luminol in Aqueous Solution, Anal. Sci., 4 (1988) 9-12.

1185. R.H. Atallah, G.D. Christian and S.D. Hartenstein, Continuous Flow Solvent Extraction System for the Determination of Trace Amounts of Uranium in Nuclear Waste Processing Solutions, Analyst, 113 (1988) 463-469.

1186. B. Karlberg, Flow Injection Extraction in Theory and Practice, Fresenius' Z. Anal. Chem., 329 (1988) 660-662.

1187. M.A.J. Van Opstal, J.S. Blauw, J.J.M. Holthuis, W.P. Van Bennekom and A. Bult, On-Line Electrochemical Derivatization Combined with Diode-Array Detection in Flow-Injection Analysis, Rapid Determination of Etoposide and Teniposide in Blood Plasma, Anal. Chim. Acta, 202 (1987) 35-47.

1188. K.H. Kroner, On-Line Determination of Enzymes in Bioprocessing with the Emphasis in Flow Injection Analysis (FIA) and Continuous Sampling, Fresenius' Z. Anal. Chem., 329 (1988) 718-725.

1189. L. Locascio-Brown, A.L. Plant and R.A. Durst, Behavior of Liposomes in Flow Injection Systems, Anal. Chem., 60 (1988) 792-797.

1190. J. Moller and A. Sjodin, Determination of Hydroxyproline in Meat Products by Flow-Injection Analysis, Fresenius' Z. Anal. Chem., 329 (1988) 732-734.

1191. J. Ruzicka, Flow Injection Analysis - a Survey of Its Potential as Solution Handling and Data Gathering Technique in Chemical Research and Industry, Fresenius' Z. Anal. Chem., 329 (1988) 653-655.

1192. P.D. Wentzell, A.P. Wade and S.R. Crouch, Modeling Chemical Response Surfaces with the Kalman Filter, Anal. Chem., 60 (1988) 905-911.

1193. C. Thommen, M. Garn and M. Gisin, Expansion of the Dynamic Range of Flow-Injection Analysis Systems for Complete Batch Process Monitoring, Fresenius' Z. Anal. Chem., 329 (1988) 678-684.

352

1194.   A.S. Attiyat, Y.A. Ibrahim, A.M. Kadry, R.Y. Xie and G.D. Christian, Flow Injection Study of the Potentiometric Lithium Selectivity of Cyclic Dioxadiamides Containing Oxygen and Nitrogen Atoms, Fresenius' Z. Anal. Chem., 329 (1987) 12-17.

1195.   K. Hool and T.A. Nieman, Immobilized Luminol Chemiluminescence Reagent System for Hydrogen Peroxide Determinations in Flowing Streams, Anal. Chem., 60 (1988) 834-837.

1196.   W.A. De Oliveira and C. Pasquini, Determination of Ethanol Using Flow Injection Enthalpimetry, Analyst, 113 (1988) 359-360.

1197.   O. Nygren, C.-A. Nilsson and A. Gustavsson, Determination of Lead in Blood Using Flow Injection and a Nebulizer Interface for Flame Atomic Absorption Spectrometry, Analyst, 113 (1988) 591-594.

1198.   H. Wada, T. Murakawa, G. Nakagawa, Simultaneous Determination of Iron and Copper Ions by Flow-Injection Analysis with a Multichannel Photo-Diode-Array Detector, Anal. Chim. Acta, 200 (1987) 515-521.

1199.   P. Linares, M.D. Luque de Castro and M. Valcarcel, Sequential Determination of Glucose and Fructose in Foods by Flow-Injection Analysis with Immobilized Enzymes, Anal. Chem. Acta, 202 (1987) 199-205.

1200.   M.A. Koupparis and P.I. Anagnostopoulou, Automated Flow Injection Determination of Salicylates Using Trinder Reaction for Clinical Analysis, Assays and Dissolution Studies of Formulations, J. Pharm. Biomed. Anal., 6 (1988) 35-46.

1201.   P.J.M. Kwakman, U.A.T. Brinkman, R.W. Frei, G.J. De Jong, F.J. Spruit, N.G.F.M. Lammers and J.H.M. Van den Berg, A Post-Column Extraction System for the Determination of Tertiary Amines by Liquid Chromatography with Chemiluminescent Detection, Chromatographia, 24 (1987) 395-399.

1202.   J. Ruz, F. Lazaro and M.D. Luque de Castro, Immobilized Enzymes in Flow-Injection Analysis: Present and Trends, J. Autom. Chem., 10 (1988) 15-19.

1203.   R.K. Bayer, Conductive PE-Carbon Composites by Elongation Flow Injection Molding. Part 1. Flow-Induced Conductivity Profile-High Molecular Weight Matrix, J. Mater. Sci., 23 (1988) 475-480.

1204.   E.M. Abdel-Moety, A.A. Moustafa, A.K.S. Ahmad and A.E. El-Gendy, Flow-Injection Analysis (FIA) of Pharmaceutical Compounds. IV. Quantification of Azintamide (Ora-Gallin) in Tablets by UV-Spectrophotometric Detection, Sci. Pharm., 55 (1987) 259-265.

1205.   A. Berthod, S.H. Brooks and J.G. Dorsey, Theoretical Study of Critical Micelle Concentration Determination by Flow Injection Analysis, J. Colloid Interface Sci., 122 (1988) 514-520.

1206.   J.R. Dean, L. Ebdon, H.M. Crews and R.C. Massey, Characteristics of Flow Injection Inductively Coupled Plasma Mass Spectrometry for Trace Metal Determination, J. Anal. At. Spectrom., 3 (1988) 349-354.

1207.   D.E. Davey and G.J.H. Metz, Determination of Copper in Concentrated Matrixes by Flow Injection with Inductively Coupled Plasma Atomic Emission Spectrometry, J. Anal. At. Spectrom., 3 (1988) 375-38.

1208. P. Vinas, M. Hernandez Cordoba and C. Sanchez-Pedreno, Determination of Nitrite by Reverse Flow Injection Analysis, Int. J. Environ. Anal. Chem., 32 (1988) 279-289.

1209. W.G. Kuhr and J. Korf, Direct Coupling of Intracerebral Dialysis with Flow Injection Analysis Based on Enzymatic/Fluorescence Detection, Anal. Chim. Acta, 205 (1988) 53-59.

1210. E.A.G. Zagatto, I.L. Mattos and A.O. Jacintho, Determination of Sucrose in Sugar-Cane Juice and Molasses by Flow-Injection Spectrophotometry, Anal. Chim. Acta, 304 (1988) 259-270.

1211. A.A. Alwarthan and A. Townshend, Determination of Tetracycline by Flow Injection with Chemiluminescence Detection, Anal. Chim. Acta, 205 (1988) 261-265.

1212. T.D. Yerian, G.D. Christian and J. Ruzicka, Flow Injection Analysis as a Diagnostic Tool for Development and Testing of a Penicillin Sensor, Anal. Chem., 60 (1988) 1250-1256.

1213. G.S. Pyen and R.F. Browner, Comparison of Flow Injection and Continuous Sample Introduction for Automatic Simultaneous Determination of Arsenic, Antimony and Selenium by Hydride Generation and ICP Optical Emission Spectrometry, Appl. Spectrosc., 42 (1988) 508-512.

1214. V.R. Villanueva and M. Mardon, Chromatography, Flow Injection Analysis and Electrophoresis in Computer-Assisted Comparative Biochemistry: Its Application and Possibilities in Clinical Research. Preliminary Studies on Crohns Disease, J. Chromatogr., 440 (1988) 261-273.

1215. J.I.F. Van Veen, M.A.J. Van Opstal, J.M. Reijn, W.P. Van Bennekom and A. Bult, Optimization Criteria for a Single Bead-String Reactor in Flow-Injection Analysis Based on Consecutive Kinetic Reactions, Anal. Chim. Acta, 204 (1988) 29-41.

1216. H.L. Nekimken, B.F. Smith, G.D. Jarvinen, E.J. Peterson and M.M. Jones, Computer Controlled Flow-Injection Analysis System for On-Line Determination of Distribution Ratios, Anal. Chem., 60 (1988) 1390-1393.

1217. T.J. Cardwell, R.W. Cattrall, P.J. Iles and I.C. Hamilton, Photocured Polymers in Ion-Selective Membranes. Part 3. A Potassium Electrode for Flow-Injection Analysis, Anal. Chim. Acta, 204 (1988) 329-332.

1218. M. Yamamoto, Y. Obata, Y. Nitta, F. Nakata and T. Kumamaru, Microporous Polytetrafluorethylene Tube Separator for the Determination of Beryllium by Flow Injection or Suction-Flow-Solvent Extraction Followed by ICP Atomic Emission Spectrometry, J. Anal. At. Spectrom., 3 (1988) 441-445.

1219. Y. Narusawa, Flow-Injection Spectrophotometric Determination of Silicate, Phosphate, and Arsenate with On-Line Column Separation, Anal. Chim. Acta, 204 (1988) 53-62.

1220. T. Yao and T. Wasa, Flow-Injection System for Simultaneous Assay of Free and Total Cholesterol in Blood Serum by Use of Immobilized Enzymes, Anal. Chim. Acta, 207 (1988) 319-323.

1221. V. Kuban and P. Dolezel, Multicomponent Determination by Flow-Injection Analysis Using the PU 4021 Diode Array Detector, Direct Determination of Lanthanides, Coll. Czech. Chem. Commun., 53 (1988) 543-553.

1222.   M.H. Memon and P.J. Worsfold, Analytical Applications of Microemulsions. Spectrophotometric Determination of Zinc Using Dithizone, Analyst, 113 (1988) 769-771.

1223.   I.R.C. Whiteside, P.J. Worsfold and E.H. McKerrell, Flow-Injection Determination of Secondary Amines in Nonaqueous Solution with Fluorescence Detection, Anal. Chim. Acta, 204 (1988) 343-348.

1224.   K. Kimura, S. Iketani, H. Sakamoto and T. Shono, Applicability of Chromogenic 14-Crown-4 Derivative to Extraction-Spectrophotometric Flow-Injection Analysis for Lithium Ion in Blood Serum, Anal. Sci., 4 (1988) 221-222.

1225.   T.D. Yerian, G.D. Christian and J. Ruzicka, Flow-Injection Analysis as a Diagnostic Technique for Development and Testing of Chemical Sensors, Anal. Chim. Acta, 204 (1988) 7-28.

1226.   M.H. Ho, Use of Immobilized Enzyme Reactors in Flow-Injection Analysis, Methods Enzymol., 137 (1988) 271-287.

1227.   D.S. Wright, M.L. Friedman, S.H. Jenkins, W.R. Heineman and H.B. Halsall, Sequestration Electrochemistry: the Interaction of Chlorpromazine and Human Orosomucoid, Anal. Biochem., 171 (1988) 290-293.

1228.   S. Motomizu, M. Onoda, M. Oshima and T. Iwachido, Spectrophotometric Determination of Potassium in River Water Based on Solvent Extraction of the Complex Formed with Crown Ether and an Anionic Azo Dye Using Flow Injection, Analyst, 113 (1988) 743-746.

1229.   R. Nakata, T. Kawamura, K. Sakashita and A. Nitta, Determination of Ammonium Ion in a Flow-Injection System with a Gas-Diffusion Membrane. Selection of Optimal Conditions for the pH Indicator, Anal. Chim. Acta, 208 (1988) 81-90.

1230.   S.E. Birnie, Automated Continuous Monitoring of Inorganic and Total Mercury in Wastewater and Other Waters by Flow-Injection Analysis and Cold-Vapor Atomic Absorption Spectrometry, J. Autom. Chem., 10 (1988) 140-143.

1231.   N.P. Evmiridis, Prospects of Suing Chemiluminescence Emission Generated During Oxidation of Pyrogallol with Periodate for the Determination of Pyrogallol with Flow Injection, Analyst, 113 (1988) 1051-1056.

1232.   H. Luedi, M. Garn, O. Ghisalba and H.P. Schaer, Enzymatic Determination of Dimethylformamide in Wastewater Using Flow Injection Analysis, Int. J. Environ. Anal. Chem., 33 (1988) 131-139.

1233.   Y. Gui, S.A. Soper and T. Kuwana, Thin-Layer Spectroelectrochemical Cuvette Cells with Long Optical Path Lengths, Anal. Chem., 60 (1988) 1645-1648.

1234.   J. Wang, T. Golden and R. Li, Cobalt Phtalocyanine/Cellulose Acetate Chemically Modified Electrodes for Electrochemical Detection in Flowing Streams, Anal. Chem., 60 (1988) 1642-1645.

1235.   B. Bouzid and A.M.G. Macdonald, Flow-Injection Spectrophotometric Determination of Aluminum Based on Chrome Azurol S and Cetylpyridinium Chloride, Anal. Chim. Acta., 207 (1988) 337-342.

1236.   J. Toei, Multifunction Valve for Flow-Injection Analysis, Analyst, 113 (1988) 731-733.

1237. C.A. Georgiou and M.A. Koupparis, Automated Flow-Injection Spectrophotometric Nonaqueous Pseudotitrations of Amines and Their Hydrohalide Salts, Analyst, 113 (1988) 755-760.

1238. Z. Dvorak, Flow-Injection Determination of Creatinine in Animal Tissues, Anal. Chim. Acta, 208 (1988) 307-312.

1239. M. Zenki, H. Komatsubara and K. Toei, Determination of Residual Chlorine in Tap Water by Flow-Injection Spectrophotometry, Anal. Chim. Acta, 208 (1988) 317-320.

1240. S.D. Kolev and E. Pungor, Mathematical Modeling of Single-Line Flow-Injection Analysis Systems without Chemical Reaction, Anal. Chem., 60 (1988) 1700-1709.

1241. J. Toei, A New Flow-Injection Titration Analysis, Fresenius' Z. Anal. Chem., 330 (1988) 484-488.

1242. J.F. Van Staden, Determination of Sulphide Using Flow-Injection Analysis with a Coated Tubular Solid-State Silver-Sulphide Ion-Selective Electrode, Analyst, 113 (1988) 885-889.

1243. Y. Israel, Theoretical and Practical Aspects of Acid-Base Determinations by Spectrophotometric Flow-Injection Analysis, Anal. Chim. Acta, 206 (1988) 313-332.

1244. W. Matuszewski and M. Trojanowicz, Graphite Paste-Based Enzymatic Glucose Electrode for Flow-Injection Analysis, Analyst, 113 (1988) 735-738.

1245. C.A. Swindlehurst and T.A. Nieman, Flow-Injection Determination of Sugars with Immobilized Enzyme Reactors and Chemiluminescence Detection, Anal. Chim. Acta, 205 (1988) 195-205.

1246. J.A. Cox, T. Gray and K.R. Kulkarni, Stable Modified Electrodes for Flow-Injection Amperometry: Application to the Determination of Thiocyanate, Anal. Chem., 60 (1988) 1710-1713.

1247. K. Kurkijarvi, P. Turunen, T. Heinonen, O. Kolhinen, R. Raunio, A. Lundin and T. Lovgren, Flow-Injection Analysis with Immobilized Chemiluminescent and Bioluminescent Columns, Methods Enzymol., 137 (1988) 171-181.

1248. B.A. Petersson, Amperometric Assay of Glucose and Lactic Acid by Flow-Injection Analysis, Anal. Chim. Acta, 209 (1988) 231-237.

1249. B.A. Petersson, Enzymic Determination of Urea in Undiluted Whole Blood by Flow-Injection Analysis Using an Ammonia Ion-Selective Electrode, Anal. Chim. Acta, 209 (1988) 239-248.

1250. W. Maher, D. Lambert, J. Furlonger and P. Cullen, Determination of Phosphorus in Biological Tissues by Aluminum Block Digestion and Flow-Injection Spectrophotometry, Anal. Chim. Acta, 209 (1988) 299-302.

1251. T. Yao, R. Akasake and T. Wasa, Simultaneous Assay of Glucose and Cholesterol in Blood Serum by Combined Use of Immobilized Enzyme Reactors and Enzyme Electrode in a Flow-Injection System, Chem. Express, 3 (1988) 275-278.

356

1252.   D. Narinesingh, D. Jaipersad and I. Chang-Yen, Immobilization of Linamarase and Its Use in the Determination of Bound Cyanide in Cassava Using Flow Injection Analysis, Anal. Biochem., 172 (1988) 89-95.

1253.   D.J. Mazzo, S.E. Biffar, K.A. Forbes, C. Bell and M.A. Brooks, A Flow Injection System with In-Series Ultraviolet and Electrochemical Detection for the Simultaneous Determination of Lovastatin and Butylated Hydroxyanisole in a Tablet, J. Pharm. Biomed. Anal., 6 (1988) 271-276.

1254.   D.B. Gladilovich, V. Kuban and L. Sommer, Determination of the Sum of Rare Earth Elements by Flow-Injection Analysis with Arsenazo III, 4-(2-Pyridylazo)resorcinol, Chrome Azurol S, and 5-Bromo-2-(2-pyridylazo)-5-diethylaminophenol Spectrophotometric Reagents, Talanta, 35 (1988) 259-265.

1255.   E.B. Milosavljevic, L. Solujic, J.H. Nelson and J.L. Hendrix, Simultaneous Flow-Injection Speciation of Iron(II) and Iron(III) Cyano Complexes Utilizing Electrochemical and Atomic Absorption Detectors in Series, Fresenius' Z. Anal. Chem., 330 (1988) 614-618.

1256.   J. Ye and R.P. Baldwin, Flow-Injection Analysis for Electroinactive Anions at a Polyanailine Electrode, Anal. Chem., 60 (1988) 1979-1982.

1257.   S.H. Brooks, R.N. Williams and J.G. Dorsey, Comparison of Aqueous, Micellar, and Microemulsion Carriers in Flow-Injection Analysis. The Base Hydrolysis of Acetylsalicylic Acid, Anal. Lett., 21 (1988) 583-598.

1258.   R.K. Force, Surface-Enhanced Raman Spectroscopy at a Silver Electrode as a Detector in Flow-Injection Analysis, Anal. Chem., 60 (1988) 1987-1989.

1259.   L. Almestrand, M. Betti, C. Hus, D. Jagner and L. Renman, Determination of Lead in Whole Blood with a Simple Flow-Injection System and Computerized Stripping Potentiometry, Anal. Chim. Acta, 209 (1988) 339-343.

1260.   J. Hernandez Mendez, R. Carabias Martinez, F. Becerro Dominguez and J.I. Jimenez Jimenez, Determination of the Pesticides Fenthion and Fenitrothion by Flow Injection with Amperometric Detection, Anal. Chim. Acta, 209 (1988) 205-212.

1261.   C.L.M. Stults, A.P. Wade and S.R. Crouch, Investigation of Temperature Effects on Dispersion in a Flow-Injection Analyzer, A Laboratory Exercise, J. Chem. Educ., 65 (1988) 645-647.

1262.   S.H. Brooks, A. Berthod, B.A. Kirsch and J.G. Dorsey, Flow-Injection System for Determination of Critical Micelle Concentrations of Ionic and Nonionic Surfactants, Anal. Chim. Acta, 209 (1988) 111-121.

1263.   B. Olsson, A Flow-Injection System Based on Immobilized Penicillinase and a Linear pH-Buffer for Potentiometric Determination of Penicillin, Anal. Chim. Acta, 209 (1988) 123-133.

1264.   B. Gallo, R.M. Alonso, F. Vicente, J.C. Vire and G.J. Patriarche, Photometric Flow Injection Determination of Psychotropic Drugs: Brotizolam and Midazolam, Anal. Lett., 21 (1988) 1211-1220.

1265.   R.C. Hutton and A.N. Eaton, Analysis of Solutions Containing High Levels of Dissolved Solids by Inductively Coupled Plasma Mass Spectrometry, J. Anal. At. Spectrom., 3 (1988) 547-550.

1266.   F. Nakata, H. Sunahara, H. Fujimoto, M. Yamamoto and T. Kumamaru, Determination of Germanium by Hydride Generation Inductively Coupled Plasma Atomic Emmision Spectrometry Combined with Flow Injection, J. Anal. At. Spectrom., 3 (1988) 579-582.

1267.   B. Olsson, G. Marko-Varga, L. Gorton, R. Appelqvist and G. Johansson, Determination of Myo-Inositol in a Flow-Injection System with Immobilized Enzyme Reactors and Amperometric Detection, Anal. Chim. Acta, 206 (1988) 49-55.

1268.   N. Yonehara, S. Akaike, H. Sakamoto and M. Kamada, Flow Injection Spectrophotometric Determination of Trace Amounts of Bromide by Its Catalytic Effect on the Hydrogen Peroxide Oxidation of Pyrocatechol Violet, Anal. Sci., 4 (1988) 273-276.

1269.   V. Kuban and D.B. Gladilovich, Simultaneous Determination of Iron and Copper in Waters by Multiligand Flow Injection Analysis, Coll. Czech. Chem. Commun., 53 (1988) 1461-1475.

1270.   T. Ohta, N. Goto and S. Takitani, Spectrophotometric Determination of N-Nitroso Compounds by Flow-Injection Analysis, Analyst, 113 (1988) 1333-1335.

1271.   S.D. Kolev and E.Pungor, Influence of the Main Parameters of Single-Line Flow-Injection Systems without Chemical Reaction on the Output Signal. Part 1. Dispersion Characteristics of the Main Sections of the Flow System, Anal. Chim. Acta, 208 (1988) 117-132.

1272.   S.D. Kolev and E. Pungor, Influence of the Main Parameters of Single-Line Flow-Injection Systems without Chemical Reaction on the Output Signal. Part 2. Simulation of the Mathematical Models, Anal. Chim. Acta, 208 (1988) 133-149.

1273.   W. Frenzel, Enhanced Performance of Ion-Selective Electrodes in Flow-Injection Analysis: NonNernstian Response, Indirect Determination, Differential Detection, and Modified Reverse Flow-Injection Analysis, Analyst, 113 (1988) 1039-1046.

1274.   T. Kuwana, Stopped-Flow Determination of the Parameters Affecting the Application of Peroxyoxylate Chemiluminescence to High-Performance Liquid Chromatographic Detection, Anal. Chem., 60 (1988) 2193-2197.

1275.   L.A. Larew, D.A. Mead Jr. and D.C. Johnson, Flow-Injection Determination of Starch and Total Carbohydrate with an Immobilized Glucoamylas Reactor and Pulsed Amperometric Detection, Anal. Chim. Acta, 204 (1988) 43-51.

1276.   G.G. Neuburger and D.C. Johnson, Pulsed Coulometric Detection with Automatic Rejection of Background Signal in Surface-Oxide-Catalyzed Anodic Detections at Gold Electrodes in Flow-through Cells, Anal. Chem., 60 (1988) 2288-2293.

1277.   G. Decristoforo, Flow-Injection Analysis for Automated Determination of ß-Lactams Using Immobilized Enzyme Reactors with Thermistor or Ultraviolet Spectrophotometric Detection, Methods Enzymol., 137 (1988) 197-217.

1278.   J.S. Canham, G.Gordon and G.E. Pacey, Optimization of Parameters for Gas Diffusion Flow Injection Systems, Anal. Chim. Acta, 209 (1988) 157-163.

358

1279.   W. Matuszewski and M. Trojanowicz, Selective Flow-Injection Determination of Residual Chlorine at Low Levels by Amperometric Detection with Two Polarized Platinum Electrodes, Anal. Chim. Acta, 207 (1988) 59-65.

1280.   C. Martinez-Lozano, T. Perez-Ruiz, V. Tomas and E. Yague, Flow Injection Determination of Oxidants with Leuco-Thionine Blue, Analyst, 113 (1988) 1057-1060.

1281.   S. Uchiyama, Y. Tofuku and S. Suzuki, Flow-Injection Determination of L-Ascorbate with Cucumber Juice as Carrier, Anal. Chim. Acta, 208 (1988) 291-294.

1282.   G.J. Moody, G.S. Sanghera and J.D.R. Thomas, Factors Concerning the Design and Calibration of an Amperometric Enzyme Electrode System for the Flow-Injection Analysis of Cholesterol, Analyst, 113 (1988) 1419-1422.

1283.   W. Davison and T.R. Harbinson, Transient Responses of Glass Electrodes in Dilute Solutions and Freshwaters, Analyst, 113 (1988) 1537-1540.

1284.   D. Prada, E. Lorenzo, M. Hernandez and L. Hernandez, Rapid Determinations of Some 1,4-Benzodiazepines by Flow Injection Analysis, Microchem. J., 37 (1988) 257-262.

1285.   H. Ji and E. Wang, Flow Injection Amperometric Detection Based on Ion Transfer Across a Water-Solidified Nitrobenzene Interface for the Determination of Tetracycline and Terramycin, Analyst, 113 (1988) 1541-1543.

1286.   J. Toei, Potential of the Flow-Gradient Function in Flow-Injection Analysis with a Multifunction Pump Delivery System, Talanta, 35 (1988) 425-430.

1287.   M. Trojanowicz, T. Krawczynski, V. Krawczyk and W. Augustyniak, Simple Antilog Converter for Conventional and Flow-Injection Measurements with Ion-Selective Electrodes, Anal. Chim. Acta, 207 (1988) 325-330.

1288.   H.A. Archontaki and S.R. Crouch, Evaluation of an Isolated Droplet Sample Introduction System for Laserinduced Breakdown Spectroscopy, Appl. Spectrosc., 42 (1988) 741-746.

1289.   M.F. Gine, F.J. Krug, H. Bergamin Filho, B.F. Reis and E.A.G. Zagatto, Flow Injection Calibration of Inductively Coupled Plasma Atomic Emission Spectrometry Using the Generalized Standard Additions Method, J. Anal. At. Spectrom., 3 (1988) 673-678.

1290.   M. Del Valle, J. Alonso, J. Bartroli and I. Marti, Spectrophotometric Determination of Low Levels of Anionic Surfactants in Water by Solvent Extraction in a Flow Injection System, Analyst, 113 (1988) 1677-1681.

1291.   P.C. Hauser, S.S. Tan, T.J. Cardwell and R.W. Cattrall, Versatile Manifold for the Simultaneous Determination of Ions in Flow Injection Analysis, Analyst, 113 (1988) 1551-1555.

1292.   R. Kuroda, T. Nara and K. Oguma, Simultaneous Determination of Iron(III) and Total Iron by Flow Injection Analysis Using Kinetic Spectrophotometry with Tiron, Analyst, 113 (1988) 1557-1560.

1293.   T.P. Lynch, Inverse Spectrophotometric Detection in Flow Injection Analysis: Determination of Nitrite Using Cerium(IV) as the Chromophore, Analyst, 113 (1988) 1597-1599.

1294. A.Y. Chamsi and A.G. Fogg, Oxidative Flow Injection Amperometric Determination of Nitrite at an Electrochemically Pre-Treated Glassy Carbon Electrode, Analyst, 113 (1988) 1723-1727.

1295. M.B. Garn, M. Gisin, H. Gross, P. King, W. Schmidt and C. Thommen, Extensive Flow-Injection Dilution for In-Line Sample Pretreatment, Anal. Chim. Acta, 207 (1988) 225-231.

1296. W.E. Van der Linden, The Application of Strongly Oxidizing Agents in Flow Injection Analysis. Part 4. Manganese(IV) and Copper(III), Anal. Chim. Acta, 211 (1988) 99-104.

1297. U. Nalbach, H. Schiemenz, W.W. Stamm, W. Hummel and M.R. Kula, On-Line Flow-Injection Monitoring of the Enzyme Inductor L-Phenylaniline in the Continuous Cultivation of Rhodococcus Sp. M4, Anal. Chim. Acta, 213 (1988) 55-60.

1298. O.F. Kamson, Flow-Injection Determination of Some Sulphur Anions via the Catalyzed Iodine-Azide Reaction, Anal. Chim. Acta, 211 (1988) 299-303.

1299. Z. Zhu and Z. Fang, Spectrophotometric Determination of Total Cyanide in Waste Waters in a Flow-Injection System with Gas-Diffusion Separation and Preconcentration, Anal. Chim. Acta, 198 (1987) 25-36.

1300. A. Farran and J. De Pablo, Continuous-Flow Extraction of Organophosphorus Pesticides Coupled On-Line with High Performance Liquid Chromatography, Anal. Chim. Acta, 212 (1988) 123-131.

1301. K. Fujiwara, Application of a Wave-Guide Capillary Cell in the Determination of Copper by Flow Injection Analysis, Anal. Chim. Acta, 212 (1988) 245-251.

1302. Y. Yuan, Simultaneous Spectrophotometric Determination of Calcium and Magnesium with Chlorophosphonazo-III by Flow Injection Analysis, Anal. Chim. Acta, 212 (1988) 291-295.

1303. M. Trojanowisc, P.J. Worsfold and J.R. Clinch, Solid-State Photometric Detectors for Flow Injection Analysis, Trends in Anal. Chem., 7 (1988) 301-305.

1304. J. Martinez Calatayud, P. Campins Falco and R. Mico Albert, Flow Method for the Titration of Weak Acids or Weak Bases Using Linear Titration Plots, Analyst, 112 (1987) 1063-1066.

1305. H. Hara, A. Motoike and S. Okazaki, Continuous Flow Determination of Low Concentrations of Ammonium Ions Using a Gas Dialysis Concentrator and a Gas Electrode Detector System, Analyst, 113 (1988) 113-115.

1306. H. Hara, A. Motoike and S. Okazaki, Alternate Washing Method for Flow-through Determination of Ammonium Ions Using a Ammonia Gas Electrode, Anal. Chem., 59 (1987) 1995-1999.

1307. H. Hara, Y. Wakizaka and S. Okazaki, Continuous Flow Determination of Chloride in the Non-Linear Response Region with a Tubular Chloride Ion-Selective Electrode, Talanta, 34 (1987) 921-926.

1308. A. Sjodin and U. Sundqvist, Advances in Flow Injection Analysis, International Laboratory, Jan/Feb. (1988) 40-49.

360

1309.   S. Motomizu, M. Oshima and T. Kuroda, Spectrophotometric Determination of Anionic Surfactants in Water after Solvent Extraction Coupled with Flow Injection, Analyst, 113 (1988) 747-753.

1310.   D.B. Gladilovich, V. Kuban and L. Sommer, Determination of Rare-Earth Elements Ion Pair HPLC with Post Column Derivatization Using Arsenazo III, Coll. Czech. Chem. Commun., 53 (1988) 1664-1677.

1311.   J. Ruz, A. Rios, M.D. Luque de Castro and M. Valcarcel, Determination of Glucose in Alcoholic Beverages by Flow Injection with Two Internally Coupled Injection Valves and an Enzyme Reactor, Anal. Chim. Acta, 211 (1988) 281-285.

1312.   H. Wada, T. Ozawa and G. Nakagawa, Preparation and Examination of Calcium Ion-Selective Electrodes for Flow Injection Analysis, Anal. Chim. Acta, 211 (1988) 213-221.

1313.   B. Bouzid and A.M.G. Macdonald, Flow-Injection Methods for the Determination of Uracil Derivatives with Voltametric Detection, Anal. Chim. Acta, 211 (1988) 175-193.

1314.   K. Schugerl, On-Line Analysis and Control of Production of Antibiotics, Anal. Chim. Acta, 213 (1988) 1-9.

1315.   L. Gorton and A. Hedlund, A Flow-Injection Method for the Amperometric Determination of L-Lactate with Immobilized Enzymes and a Chemically Modified Electrode, Anal. Chim. Acta, 213 (1988) 91-100.

1316.   E. Dominguez and B. Hahn-Hagerdahl, A Flow-Injection System for the Amperometric Determination of Xylose and Xylulose with Co-Immobilized Enzymes and a Modified Electrode, Anal. Chim. Acta, 213 (1988) 139-150.

1317.   L.F. Capitan-Vallvey, M.C. Valencia and J.M. Bosque Sendra, Microdetermination of Fluoride in Water by Ion-Exchange Spectrophotometry, Analyst, 113 (1988) 419-422.

1318.   D.B. Gladilovich and V. Kuban, Spectrophotometric Determination of Lanthanoides and Yttrium by Flow Injection Analysis, Using Chrome Azurol S in the Presence of Cationic Surfactants, Chem. Papers, 42 (1988) 607-620.

1319.   P.K. Tarafder and D.P.S. Rathore, Spectrophotometric Determination of Nitrite in Water, Analyst, 113 (1988) 1073-1076.

1320.   A.J. Kempers and A.G. Luft, Re-Examination of the Determination of Environmental Nitrate by Reduction with Hydrazine, Analyst, 113 (1988) 1117-1120.

1321.   M. Drikas and B.I. Routley, Spectrophotometric Method for the Determination of Total Cyanide in Wastewater Samples, Analyst, 113 (1988) 1273-1276.

1322.   F. Sanchez Rojas, A. Garcia de Torres, C. Bosch Ojeda and J.M. Cano Pavon, N-(3-Hydroxy-2-pyridyl)-salicylaldimine as a Reagent for the Selective Fluorimetric Determination of Aluminum in Waters, Analyst, 113 (1988) 1287-1290.

1323.   A.E. Villa, Rapid Method for Determining Very Low Fluoride Concentrations Using an Ion-Selective Electrode, Analyst, 113 (1988) 1299-1303.

1324. A.G. Fogg and A.B. Ghawji, Reductive Amperometric Determination of Nitrofurantoin and Acetazolamide at a Sessile Mercury Drop Electrode Using Flow Injection Analysis, Analyst, 113 (1988) 727-730.

1325. S. Girotti, A. Roda, M.A. Angellotti, S. Ghini, G. Carrea, R. Bovara, S. Piazzi and R. Merighi, Bioluminescence Flow System for the Determination of Branched-Chain L-Amino Acids in Serum and Urine, Anal. Chim. Acta, 205 (1988) 229-237.

1326. K. Yoshimura, Application of Ion-Exchanger Phase Absorptiometry to Flow Analysis, Determination of Trace Amounts of Chromium(IV) in Water, Analyst, 113 (1988) 471-474.

1327. R.E. Sioda, Parabolic Flow Velocity Distribution in Electrolysis with Flow through Electrodes, Analyst, 113 (1988) 489-492.

1328. C. Gutierrez, A. Gomez-Hens and D. Prez-Bendito, Stopped-Flow Fluorimetric Determination of Theophylline in Pharmaceutical Preparations, Analyst, 113 (1988) 559-562.

1329. A. Yuchi, N. Yanai, H. Wada and G. Nakagawa, Tiron as a Masking Agent for Aluminum in the Determination of Fluoride with an Ion-Selective Electrode: Equilibrium Studies and Application, Analyst, 113 (1988) 1405-1408.

1330. A.C. Chang, G.-C. Lin and D.E. Moss, Determination of Methanesulphonyl Fluoride in Blood Matrix with a Fluoride Ion-Selective Electrode, Analyst, 113 (1988) 1485-1486.

1331. A.M.Y. Jaber, G.J. Moody and J.D.R. Thomas, Studies on Lead Ion-Selective Electrodes Based on Polyalkoxylates, Analyst, 113 (1988) 1409-1413.

1332. R.H. Atallah, J. Ruzicka and G.D. Christian, Continuous Solvent Extraction in a Closed-Loop System, Anal. Chem., 59 (1987) 2909-2914.

1333. K. Yoshimura, Implementation of Ion Exchanger Absorptiometric Detection in Flow Analysis Systems, Anal. Chem., 59 (1987) 2922-2924.

1334. R.K. Kobos, J.W. Eveleigh, M.L. Stepler, B.J. Haley and S.L. Papa, Fluorocarbon Based Immobilization Method for Preparation of Enzyme Electrodes, Anal. Chem., 60 (1988) 1996-1998.

1335. H. Huiliang, D. Jagner and L. Renman, Flow Potentiometric and Constant-Current Stripping Analysis for Silver(I) with Carbon- and Platinum-Fibre Electrodes, Anal. Chim. Acta, 207 (1988) 27-35.

1336. M.A. Koupparis and P.I. Anagnostopoulou, Automated Flow-Injection Determination of Sulfonamides by the Bratton-Marshall Reaction for Clinical Analysis, Assays and Dissolution Studies of Formulations, Anal. Chim. Acta, 204 (1988) 271-283.

1337. N. Grekas and A.C. Calokerinos, Continuous-Flow Molecular Emission Cavity Analysis for Organic Sulphur Compounds by Alkaline Hydrolysis, Anal. Chim. Acta, 204 (1988) 285-293.

1338. F. Canete, M.D. Luque de Castro and M. Valcarcel, Determination of pH, Conductivity, Residual Chlorine and Ammonium and Nitrite Ions in Water with an Unsegmented Flow Configuration, Analyst, 113 (1988) 739-742.

362

1339.   B.F. Reis, M.A.Z. Arruda and E.A.G. Zagatto, An Improved Monosegmented Continuous-Flow System for Sample Introduction in Flame Atomic Spectrometry, Anal. Chim. Acta, 206 (1988) 253-262.

1340.   B.T. Tay, K.P. Ang and H. Gunasingham, Platinum-Dispersed Nafion-Modified Glassy Carbon Electrode for the Determination of Hydrogen Peroxide in a Flow Injection System, Analyst, 113 (1988) 617-620.

1341.   H. Gunasingham, Analytical Applications of the Wall-Jet Detector, Trends in Anal. Chem., 7 (1988) 217-221.

1342.   P. Dolezel and V. Kuban, The Determination of Cu, Ni and Co Using 2-(2-Pyridylazo)-1-naphtol-4-sulphonic Acid by Multicomponent Flow Injection Analysis (MC-FIA), Coll. Czech. Chem. Commun., 53 (1988) 1162-1171.

1343.   Y. Narusawa, T. Katsura and F. Kato, Simultaneous Determination of Silicon and Phosphorus in Biological Standard Materials with On-Line Column Flow-Injection Spectrophotometry, Fresenius' Z. Anal. Chem., 332 (1988) 162-166.

1344.   J. Ruzicka and E.H. Hansen, Homogeneous and Heterogeneous Systems, Flow Injection Analysis Today and Tomorrow, Anal. Chim. Acta, 214 (1988) 1-28.

1345.   Z. Fang, Z. Zhu, S. Zhang, S. Xu, L. Guo and L. Sun, On-Line Separation and Preconcentration in Flow Injection Analysis, Anal. Chim. Acta, 214 (1988) 41-56.

1346.   J.F. Tyson, Atomic Spectrometry and Flow Injection Analysis: a Synergic Combination, Anal. Chim. Acta, 214 (1988) 57-76.

1347.   I.C. Van Nugteren-Osinga, M. Bos and W.E. Van der Linden, Impulse/Response Functions of Individual Components of Flow Injection Manifolds, Anal. Chim. Acta, 214 (1988) 77-86.

1348.   T. Korenaga and K.K. Stewart, Seeking High-Sensitivity Flow Injection Analysis, Anal. Chim. Acta, 214 (1988) 87-96.

1349.   T. Korenaga, F.-H. Shen, H. Yoshida, T. Takahashi, Study of Sample Dispersion in Flow Injection Analysis, Anal. Chim. Acta, 214 (1988) 97-106.

1350.   G. Marko-Varga, High-Performance Liquid Chromatography Separation of Some Mono- and Disaccharides with Detection by a Postcolumn Enzyme Reactor and a Chemically Modified Electrode, J. Chrom., 408 (1987) 157-170.

1351.   G. Schultze, C.Y. Liu, M. Brodowski, O. Elsholz, W. Frenzel and J. Moller, Different Approaches to the Determination of Ammonium Ions at Low Levels by Flow Injection Analysis, Anal. Chim. Acta, 214 (1988) 121-136.

1352.   K. Nikolajsen, J. Nielsen and J. Villadsen, In-Line Flow Injection Analysis for Monitoring Lactic Acid Fermentations, Anal. Chim. Acta, 214 (1988) 137-146.

1353.   M.T. Jeppesen and E.H. Hansen, Determination of Creatinine in Undiluted Blood Serum by Enzymatic Flow Injection with Optosensing, Anal. Chim. Acta, 214 (1988) 147-160.

1354. A.M. Almuaibed and A. Townshend, Enzymatic Methods for the Determination of Ethanol Based on Linear and Cyclic Flow-Injection Systems, Anal. Chim. Acta, 214 (1988) 161-172.

1355. M. Masoom, Immobilized Enzymes in Clinical and Biochemical Analysis, Applications to the Simultaneous Determination of Acetylcholine and Choline and to the Determination of Lipids, Anal. Chim. Acta, 214 (1988) 173-186.

1356. T. Yamane, Determination of Manganese at Trace Levels in Natural Waters with Continuous Flow System Utilizing On-Line Cation-Exchange Separation and Catalytic Detection, Anal. Sci., 2 (1986) 191-195.

1357. D.A. Whitman, G.D. Christian and J. Ruzicka, Novel Use of Doublet Peaks in Flow Injection Analysis, Simultaneous Spectrophotometric Determination of Nickel(II) and Iron(II), Anal. Chim. Acta, 214 (1988) 197-206.

1358. K.K. Verma and K.K. Stewart, Spectrophotometric Determination of Aromatic Primary Amines and Nitrite by Flow Injection Analysis, Anal. Chim. Acta, 214 (1988) 207-216.

1359. F. Lazaro, M.D. Luque de Castro and M. Valcarcel, Integrated Reaction/Spectrophotometric Detection in Unsegmented Flow Systems, Anal. Chim. Acta, 214 (1988) 217-220.

1360. M. Aoyagi, Y. Yasumasa and A. Nishida, Rapid Spectrophotometric Determination of Total Phosphorus in Industrial Waste Waters by Flow Injection Analysis Including a Capillary Digestor, Anal. Chim. Acta, 214 (1988) 229-238.

1361. L.C.R. Pessenda, A.O. Jacintho and E.A.G. Zagatto, Catalytic Determination of Molybdenum in Plants by Flow Injection Spectrophotometry with Ion-Exchange Separation, Anal. Chim. Acta, 214 (1988) 239-246.

1362. I.L. Mattos, E.A.G. Zagatto and A.O. Jacintho, Spectrophotometric Flow-Injection Determination of Sucrose and Total Reducing Sugar in Sugar-Cane Juice and Molasses, Anal. Chim. Acta, 214 (1988) 247-258.

1363. A. Tanak, K. Mashiba and T. Deguchi, Simultaneous Determination of Cyanide and Thiocyanate by the Pyridine/Barbituric Acid Method after Diffusion through a Microporous Membrane, Anal. Chim. Acta, 214 (1988) 259-270.

1364. T. Sakai and N. Ohno, Flow-Injection Spectrophotometric Determination of Palladium in Catalysts and Dental Alloys with 2-(5-Bromo-s-pyridylazo)-5-(N-propyl-N-sulfopropyl-amino)aniline, Anal. Chim. Acta, 214 (1988) 271-278.

1365. X. Wu, W. Qi, Spectrophotometric Determination of Trace Uranium in Geological Samples by Flow Injection Analysis with On-Line Levextrel Resin Separation and Preconcentration, Anal. Chim. Acta, 214 (1988) 279-288.

1366. S. Motomizu and M. Onoda, Flow-Injection Determination of Sodium and Potassium by Separation on a Silica Column and Extraction-Spectrophotometry with Benzo-18-crown-6 and Tetrabromophenolphtalein Ethyl Ester, Anal. Chim. Acta, 214 (1988) 289-298.

1367. R.G. Melcher, Flow-Injection Determination of Membrane-Selected Organic Compounds, Anal. Chim. Acta, 214 (1988) 299-314.

1368.   S.J. Gluck, Determination of Water/Octanol Partition Coefficients by Flow Injection Analysis, Anal. Chim. Acta, 214 (1988) 315-328.

1369.   S.R. Bysouth, J.F. Tyson and P.B. Stockwell, Lead Preconcentration with Flow Injection for Flame Atomic Absorption Spectrometry, Anal. Chim. Acta, 214 (1988) 329-338.

1370.   C.E. Adeeyinwo and J.F. Tyson, Flow-Injection Techniques for the Removal of Stable-Compound Interferences in Flame Atomic Absorption Spectrometry of Calcium, Anal. Chim. Acta, 214 (1988) 339-348.

1371.   N. Ishibashi, T. Imato, H. Ohura and S. Yamasaki, Potentiometric Flow-Injection Determination of Trace Chlorine Based on Its Redox Reaction with an Iron(III)/Iron(II) Buffer, Anal. Chim. Acta, 214 (1988) 349-358.

1372.   T.J. Cardwell, R.W. Cattrall, P.C. Hauser, I.C. Hamilton, A Multi-Ion Sensor Cell and Data-Acquisition System for Flow Injection Analysis, Anal. Chim. Acta, 214 (1988) 359-366.

1373.   M.L. Balconi, F. Sigon, R. Ferraroli and F. Realini, Applications of Flow Injection Analysis in a Power Plant Determination of pH, Ammonia and Hydrazine in an AVT-Conditioned Water Steam Cycle, Anal. Chim. Acta, 214 (1988) 367-374.

1374.   F. Canete, A. Rios, M.D. Luque de Castro and M. Valcarcel, Simultaneous Determination of Phenolic Compounds in Water by Normal and Derivative Flow Injection/Cyclic Voltammetry, Anal. Chim. Acta, 214 (1988) 375-384.

1375.   J.S. Canham and G.E. Pacey, Flow Injection Systems for Sample Introduction in Mass Spectrometry, Anal. Chim. Acta, 214 (1988) 385-390.

1376.   K.A. McGowan and G.E. Pacey, Initial Studies of Large-Bore Flow-Injection Systems, Anal. Chim. Acta, 214 (1988) 391-396.

1377.   H. Bergamin Filho, F.J. Krug, B.F. Reis, J.A. Nobrega, M. Mesquita and I. G. Souza, On-Line Electrolytic Dissolution of Alloys in Flow Injection Analysis. Part 2. Spectrophotometric Determination of Molybdenum in Steels, Anal. Chim. Acta, 214 (1988) 397-400.

1378.   J.R. Clinch, P.J. Worsfold and F.W. Sweeting, An Automated Spectrophotometric Field Monitor for Water Quality Parameters. Determination of Ammonia, Anal. Chim. Acta, 214 (1988) 401-408.

1379.   I. Satoh and T. Ishii, Flow-Injection Determination of Inorganic Pyrophosphate with Use of an Enzyme Thermistor Containing Immobilized Inorganic Pyrophosphate, Anal. Chim. Acta, 214 (1988) 409-414.

1380.   I. Satoh, S. Arakawa and A. Okamoto, Flow-Injection Determination of Glutathione with Amperometric Monitoring of the Enzymatic Reaction, Anal. Chim. Acta, 214 (1988) 415-420.

1381.   M. Burguera, J.L. Burguera and O.M. Alarcon, Determination of Zinc and Cadmium in Small Amounts in Biological Tissues by Microwave-Assisted Digestion and Flow Injection Atomic Absorption Spectrometry, Anal. Chim. Acta, 214 (1988) 421-428.

1382.   J.L. Burguera and M. Burguera, Determination of Sulphur Dioxide in Young White Wines by Flow Injection with Chemiluminescence Detection, Anal. Chim. Acta, 214 (1988) 429-432.

1383.   T.J. Cardwell, R.W. Cattrall, M. Mitri and I.C. Hamilton, Flow-Injection Spectrophotometric Determination of Fluoride by Using the Zirconium/Alizarin Red S Complex, Anal. Chim. Acta, 214 (1988) 433-438.

1384.   H. Wada, K. Asakura, G. Venkata Rattaiah and G. Nakagawa, Preconcentration and Determination of Calcium and Magnesium Brine with Flow-Injection Systems, Anal. Chim. Acta, 214 (1988) 439-446.

1385.   J.F. Tyson and A.B. Marsden, Factors Affecting the Detection Limit of a Flow-Injection Spectrophotometric Procedure, Anal. Chim. Acta, 214 (1988) 447-454.

1386.   M. Akiba and S. Motomizu, Flow-Injection Spectrophotometric Determination of Glucose in Blood Plasma with Bindschedler's Green Leuco Base as Color Reagent, Anal. Chim. Acta, 214 (1988) 455-462.

1387.   C.F. Mandenius, In-Situ Ethanol Probe Based on Sample Dilution in a Double-Membrane System, Anal. Chim. Acta, 215 (1988) 71-77.

1388.   H.C. Smit and P.J.H. Scheeren, Characterization of Responses to Injection of Microliter Samples and to Peak-Shaped Input Signals for Inductively-Coupled Plasma/Atomic Emission Spectrometry, Anal. Chim. Acta, 215 (1988) 143-153.

1389.   P. Martinez-Jimenez, M. Gallego and M. Valcarcel, Indirect Atomic Absorption Spectrometric Determination of Some Cationic Surfactants by Continuous Liquid/Liquid Extraction with Tetrathiocyanatocobaltate, Anal. Chim. Acta, 215 (1988) 233-240.

1390.   R. Montero, M. Gallego and M. Valcarcel, Indirect Atomic Absorption Spectrometric Determination of Local Anaesthetics in Pharmaceutical Preparations with a Flow-Injection Precipitation Technique, Anal. Chim. Acta, 215 (1988) 241-248.

1391.   P.K. Dasgupta, R.S. Vithanage and K. Petersen, Pulsed Reagent Introduction through a Membrane Reactor for Flow-Injection Systems, Anal. Chim. Acta, 215 (1988) 277-282.

1392.   E. Figuerola, A. Florido, M. Aguilar, J. De Pablo and S. Alegret, Sequential Flow-Injection Determination of Cyanide and Weak Metal-Cyanide Complexes with Flow-through Heterogeneous Membrane Electrodes, Anal. Chim. Acta, 215 (1988) 283-288.

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